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(54) Vascular endothelial cell growth factor C subunit

(57) Vascular endothelial cell growth factor C subunit DNA is prepared by polymerase chain reaction techniques. The DNA encodes a protein that may exist as either a heterodimer or homodimer. The protein is a mammalian vascular endothelial cell mitogen and as such is useful for the promotion of vascular development and repair. This unique growth factor is also useful in the promotion of tissue repair.

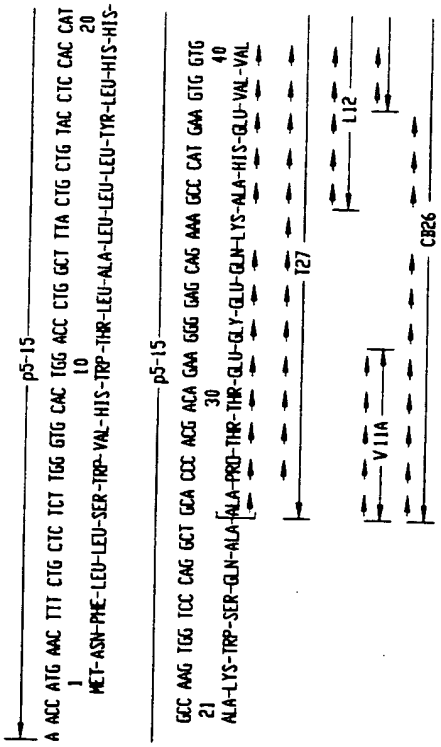

FIG.1A

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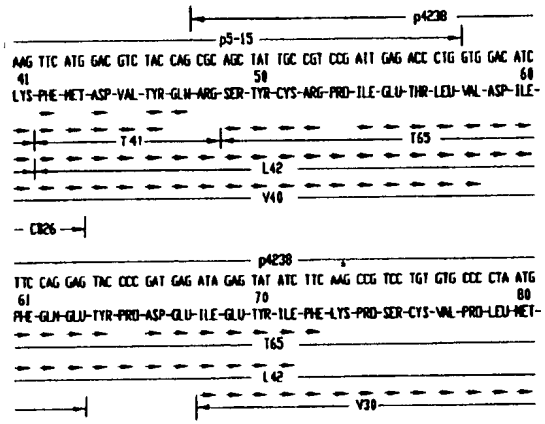

FIG.1B

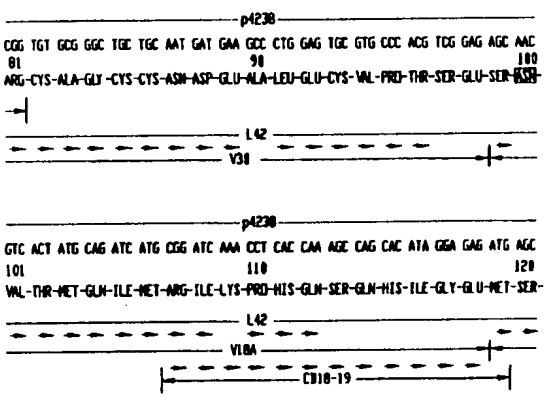

FIG.1C

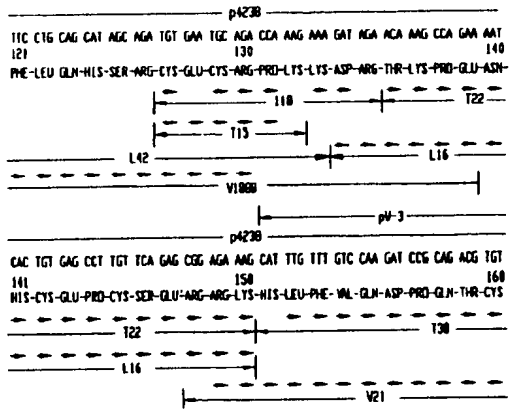

FIG.1D

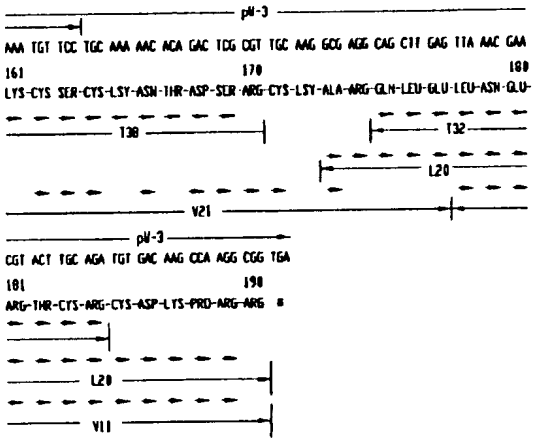

FIG.1E

BRIEF DESCRIPTION OF THE DRAWING

Figure 1. Full length amino acid residue protein translation product and its cDNA coding sequence for VEGF AA subunit A plus polypeptide cleavage products used to determine the amino acid sequence.

Figure 2. Full length amino acid residue protein translation product and its cDNA coding sequence for VEGF AB subunit A plus polypeptide cleavage products used to determine the amino acid sequence.

Figure 3. Full length amino acid residue protein translation product and its cDNA coding sequence for VEGF AB subunit B plus polypeptide cleavage products used to determine the amino acid sequence.

Figure 4. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF A 146 amino acid residue subunit SEQ ID NOS:23 & 33.

Figure 5. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF A 190 amino acid residue subunit SEQ ID NOS:30 & 31.

Figure 6. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF A 214 amino acid residue subunit SEQ ID NOS:34 & 35.

Figure 7. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF B 138 amino acid residue subunit SEQ ID NOS:36 & 37.

Figure 8. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF B 158 amino acid residue subunit SEQ ID NOS:38 & 39.

Figure 9. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF C 154 amino acid residue subunit SEQ ID NOS:40 & 41.

BACKGROUND OF THE INVENTION

A new class of cell-derived dimeric mitogens with apparently restricted specificity for vascular endothelial cells has recently been identified and generally designated vascular endothelial growth factors (VEGFs). The mitogen has been purified from: conditioned growth media of rat glioma cells, [Conn et al., Proc. Natl. Acad. Sci. USA 87: 1323-1327 (1990)]; conditioned growth media of bovine pituitary folliculo stellate cells [Ferrara and Henzel, Biochem. Biophys. Res. Comm. 161: 851-858 (1989) and Gospodarowicz et al., Proc. Natl. Acad. Sci. USA 86: 7311-7315 (1989)]. An endothelial cell growth factor isolated form mouse neuroblastoma cell line NB41 with an unreduced molecular mass of 43-51 kDa and a reduced mass of 23-29 kDa has been described by Levy et al., Growth Factors 2: 9-19 (1989). Connolly et al. (J. Biol. Chem. 264: 20017-20024 [1989]; J. Clin. Invest. 84: 1470-1478 [1989]) describe a human vascular permeability factor that stimulates vascular endothelial cells to divide in vitro and promotes the growth of new blood vessels when administered into healing rabbit bone grafts or rat corneas. An endothelial cell growth factor has been purified from the conditioned medium of the AtT-20 pituitary cell line by Plouet et al., EMBO Jownal 8: 3801-3806 (1989). The growth factor was characterized as a heterodimer composed of subunits with molecular mass of 23 kDa. Leung et al. (Science 246: 1306-1309 [1989]), Keck et al. (Science 246: 1309-1312 [1989]) and Conn et al. (Proc. Natl. Acad. Sci USA 87: 2628-2632 [1990]) have described cDNAs which encode VEGF A which is homologous to the A and B chains of platelet-derived growth factor. Vascular endothelial growth factor I (VEGF I, VEGF AA) is a homodimer with an apparent molecular mass of 46 kDa, with each subunit having an apparent molecular mass of 23 kDa. VEGF I has distinct structural similarities to platelet-derived growth factor (PDGF), a mitogen for connective tissue cells but not vascular endothelial cells from large vessels.

OBJECTS OF THE INVENTION

It is, accordingly, an object of the present invention to provide novel vascular endothelial growth factor C subunit DNA free of other mammalian DNA. Another object is to provide recombinant genes capable of expressing VEGF C subunit monomer or dimer. Another object is to provide vectors containing the DNA sequences for VEGF A or B plus C subunits. A further object is to provide a host cell transformed with a vector containing the DNA sequence for VEGF A or B plus C or VEGF C alone. It is also an object to provide a recombinant process for making VEGF C subunit. Another object is to provide a novel vascular endothelial cell growth factor which contains the C subunit. This may include heterodimers AC and BC and homodimer CC.

SUMMARY OF THE INVENTION

Vascular endothelial cell growth factor C subunit DNA is prepared by polymerase chain reaction techniques. The DNA encodes a protein that may exist as either a heterodimer or homodimer. The protein is a mammalian vascular endothelial cell mitogen and as such is useful for the promotion of vascular development and

repair. This unique growth factor is also useful in the promotion of tissue repair.

DETAILED DESCRIPTION

The present invention relates to a unique vascular endothelial cell growth factor (designated VEGF), isolated and purified from glioma cell conditioned medium, which exhibits mitogenic stimulation of vascular endothelial cells. Glioma is defined herein as any neoplasm derived from one of the various types of cells that form the interstitial tissue of the central nervous system including brain, spinal cord, posterior pituitary gland and retina. Consequently, the scope of the present invention is intended to include the unique growth factor isolated and purified from any mammalian tissue or other cells including cell lines. Cell lines include, but are not limited to, glioma-derived cell lines such as C6, hs 683 and GS-9L; glioblastomas such as A-172 and T98G; neuroblastomas such as IMR-32 and SK-N-MC; neurogliomas such as H4; tetromas such as XB-2; astrocytomas such as U-87 MG and U-373 MG; embryonal carcinomas and non-transformed glial or astrocyte cell lines, and the human medulloblastoma line TE 671, with GS-9L and TE 671 being preferred. VEGF AB is present and can be isolated from rat tissue including ovary, heart and kidney. Anterior pituitary tumor cell lines such as GH3 and Hs 199 may also be used. It is intended that VEGF of this invention can be obtained from any mammal species capable of producing VEGF, this includes, but is not limited to, rat and human.

Vascular endothelial cell growth factor may exist in various microheterogeneous forms which are isolated from one or more of the various cells or tissues described above. Microheterogeneous forms as used herein refer to a single gene product, that is a peptide produced from a single gene unit of DNA, which is structurally modified at the mRNA level or following translation. Peptide and protein are used interchangeably herein. The microneterogeneous forms will all have similar mitogenic activities. Biological activity and biologically active are used interchangeably and are herein defined as the ability of VEGF to stimulate DNA synthesis in target cells including vascular endothelial cells as described below which results in cell proliferation. The modifications may take place either in vivo, or during the isolation and purification process. In vivo modification results from, but is not limited to, proteolysis, glycosylation, phosphorylation, deamidation or acetylation at the N-terminus. Proteolysis may include exoproteolysis wherein one or more terminal amino acids are sequentially, enzymatically cleaved to produce microheterogeneous forms which have fewer amino acids than the original gene product. Proteolysis may also include endoproteolytic modification that results from the action of endoproteases which cleave the peptide at specific locations within the amino acid sequence. Similar modifications can occur during the purification process which also results in production of microheterogeneous forms. The most common modification occurring during purification is proteolysis which is generally held to a minimum by the use of protease inhibitors. Under most conditions one or more microheterogeneous forms are present following purification of native VEGFs. Native VEGFs refers to VEGF isolated and purified from cells that produce VEGFs. Vascular endothelial cell growth factor may also exist in various alternatively spliced forms which is defined herein as the production of related mRNAs by differential processing of exons and introns. Exons are defined as those parts of the DNA sequence of a eukaryotic gene that code for the final protein product. It is also intended that the present invention includes VEGF subunits A,B and C which are defined as comprising the full length translation products of all alternatively spliced mRNAs made from the gene encoding the subunits and their corresponding mature amino acid sequences generated by proteolytic removal of the amino terminal secretory leader amino acid sequences. It is further intended that the invention only include those microheterogeneous and alternatively spliced VEGF subunits which when in the dimeric form exhibit biological activity such as vascular endothelial cell stimulation as discussed below.

Glioma cells such as the rat cell line GS-9L are grown to confluence in tissue culture flasks, about 175 cm^2, in a cell culture medium such as Dulbecco's Modified Eagle's Medium (DMEM) supplemented with about 10% newborn calf serum (NCS). When the cells reach confluence the culture medium is removed, the cell layers are washed with Ca^{++}, Mg^{++}-free phosphate buffered saline (PBS) and are removed from the flasks by treatment with a solution of trypsin, about 0.1%,and EDTA, about 0.04%. The cells, about 1 x 10^8, are pelleted by centrifugation, resuspended in about 1500 ml of DMEM containing about 5% NCS and plated into a ten level cell factory (NUNC), 6,000 cm^2 surface area. The cells are incubated for about 48 to about 96 hours, with 72 hours preferred, at about 37° C in an atmosphere of about 5% CO_2. Following incubation the medium is removed and the cell factories are washed about 3 times with PBS. About 1500 ml of fresh culture media is added containing about a 1:2 mixture of Ham's-F12/DMEM containing about 15 mM Hepes, pH about 7.4, about 5 µg/ml insulin, about 10 µg/ml transferrin and with or without about 1.0 mg/ml bovine serum albumin. This medium is replaced with fresh medium after about 24 hr and collected every 48 hr thereafter. The collected conditioned medium is filtered through Whatmen #1 paper to remove cell debris and stored at about -20° C.

The GS-9L conditioned medium is thawed and brought to pH 6.0 with 1 M HCl. The initial purification step consists of cation exchange chromatography using a variety of cation exchangers on a variety of matrices such

as CM Sephadex C-50, Pharmacia Mono S, Zetachrom SP and Polyaspartic Acid WCX (Nest Group) with CM Sephadex C-50 (Pharmacia) being preferred. The VEGF-containing culture medium is mixed with CM Sephadex C-50 at about 2 gm per about 20 L of the conditioned medium and stirred at low speed for about 24 hr at 4° C. The resin is allowed to settle and the excess liquid is removed. The resin slurry is packed into a column and the remaining culture medium is removed. Unbound protein is washed from the column with 0.05 M sodium phosphate, about pH 6.0, containing 0.15 M NaCl. The VEGF AB is eluted with about 0.05 M sodium phosphate, about pH 6.0, containing about 0.6 M NaCl.

The active fractions collected from the CM Sephadex C-50 column are further fractionated by lectin affinity chromatography for additional purification of VEGF AB. The lectins which may bind VEGF AB include, but are not limited to, lectins which specifically bind mannose residues such as concanavalin A and lens culinaris agglutinin, lectins which bind N-acetylglucosamine such as wheat germ agglutinin, lectins that bind galactose or galactosamine and lectins which bind sialic acids, with concanavalin A (Con A) being preferred. A 0.9 cm diameter column containing about 5 ml packed volume of Con A agarose (Vector Laboratories) is washed and equilibrated with about 0.05 M sodium acetate, about pH 6.0, containing about 1 mM $CaCl_2$, about 1 mM $MnCl_2$ and about 0.6 M NaCl. The unbound protein is washed from the column with equilibration buffer. The VEGF AB is eluted with about 0.1 M NaCl buffer containing about 0.32 M α-methyl mannoside and about 0.28 M α-methyl glucoside.

The VEGF AB active eluate from the Con-A column is applied to a Polyaspartic Acid WCX cation exchange high performance liquid chromatography (HPLC) column, 4.6 mm x 250 mm, pre-equilibrated in about 0.05 M sodium phosphate buffer, pH 6.0. The column is eluted with a linear gradient of about 0 to 0.75 M NaCl in the phosphate buffer over about 60 minutes. The flow rate is maintained at about 0.75 ml/min collecting 0.75 ml fractions. Vascular endothelial cell growth factor AB activity is present in fractions eluting between approximately 21.7 and 28.5 ml.

The active fractions eluted from the polyaspartic WCX column that contain VEGF AB are pooled, adjusted to about pH 7.0 and loaded onto a 1 x 10 cm column of Pharmacia Chelating Sepharose 6B charged with an excess of copper chloride and equilibrated in about 0.05 M sodium phosphate, about pH 7.0, containing about 2 M NaCl and about 0.5 mM imidazole (A buffer). VEGF AB is eluted from the column with a gradient from 0-20% B over 10 minutes, 20-35% B over 45 minutes and 35-100% B over 5 minutes at a flow rate of 0.3 ml/min, where B buffer is 0.05 M sodium phosphate, pH 7.0, containing about 2 M NaCl and 100 mM imidazole. The active fractions containing VEGF AB activity eluted between about 12.6 and 22.8 ml of the gradient effluent volume.

The pooled fractions containing VEGF AB activity eluted from the metal chelate column are loaded onto a 4.6 mm x 5 cm Vydac
C_4 reverse phase HPLC column (5 μm particle size) previously equilibrated in solvent A [0.1% trifluoroacetic acid (TFA)]. The column is eluted with a linear gradient of about 0 to 30% solvent B over 15 minutes, 30% B for an additional 15 minutes, then 30-45% B over 22.5 minutes and finally 45-100% B over 5.5 minutes. Solvent B consists of solvent A containing 67% acetonitrile (v/v). The bow rate is maintained at about 0.75 ml/min and fractions are collected every minute. The homogeneous VEGF AB elutes from the C_4 column under these conditions at between about 32 and about 38 ml of the gradient effluent volume.

Purity of the protein is determined by sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (PAGE) in 12.5% crosslinked gels using the technique of Laemmli, Nature 227:680-684 (1970). The silver stained gels show VEGF AB to consist of one band under non-reducing conditions with an approximate apparent molecular mass of about 58,000 daltons. When a sample containing the microheterogeneous forms of VEGF AB is separated under reducing conditions it migrates as two about 23 kilodalton (kDa) subunits. The purification process results in VEGF AB that is essentially free of other mammalian cell products, such as proteins. Recombinantly derived VEGF AB will also be free of mammalian cell products.

Biological activity is determined by mitogenic assay using mammalian vascular endothelial cells. Human umbilical vein endothelial (HUVE) cells are plated on gelatin-coated dishes at a density of about 5000 cells per well in about 500 μl of Medium 199 (M199) containing about 20% heat-inactivated fetal calf serum (FCS). Samples to be assayed are added at the time of plating. The tissue culture plates are incubated at about 37° C for about 12 hours and about 2 microcuries of tritiated thymidine (NEN, 20 Ci/mmol) is added per ml of assay medium (1.0 μCi/well). The plates are incubated for a further 60 hr, the assay medium is removed and the plates are washed with Hanks balanced salt solution containing about 20 mM Hepes, about pH 7.5, and about 0.5 mg/ml bovine serum albumin. The cells are lysed and the labelled DNA solubilized with about 200 μl of a solution containing about 2 gm of sodium carbonate and about 400 mg sodium hydroxide in about 100 ml water. The incorporated radioactivity was determined by liquid scintillation counting. The concentration of VEGF which elicited a half-maximal mitogenic response in HUVE cells was approximately 2 ± 1 ng/ml. The glycosaminoglycan heparin, which is required in these assays at a level of 10-100 μg/ml to promote a response to a positive control, acidic fibroblast growth factor, does not enhance mitogenic stimulation of these cells by VEGF AB.

A purified about 1-2 µg sample of VEGF AB is reduced in about 0.1 M Tris, about pH 9.5, with about 0.1% EDTA, about 6 M guanidinium chloride and about 20 mM dithiothreitol for about 2 hr at about 50°C. The reduced protein is carboxymethylated for about 1 hour in a solution containing about 9.2 µM of unlabelled and 2.8 µM of ^{14}C-iodoacetic acid in about 0.7 M Tris, about pH 7.8, and about 0.1% EDTA and about 6 M guanidinium chloride. The protein is carboxymethylated for about 1 hr at room temperature. The protein is isolated after reduction and carboxymethylation by reverse phase HPLC chromatography on a Vydac C_4 column, about 4.6 mm x 5 cm. The protein subunits are loaded onto a column pre-equilibrated with about 0.1% TFA and eluted by a 45 ml linear gradient from about 0.1% TFA to 0.1% TFA/67% acetonitrile at a bow rate of about 0.75 ml/min. The reduced and carboxymethylated protein eluted as two peaks at approximately 23 and 25 ml with the proportion being approximately equal as determined by monitoring absorbance at 210 nm.

Samples of the reduced and carboxymethylated monomers are applied to polybrene-coated glass fiber filters and their N-terminal sequences are determined by Edman degradation in an ABI gas phase microsequencer in conjunction with an ABI 120A on line phenylthiohydantoin analyzer following the manufacturers instructions. The protein showing the peak of absorbance eluting at approximately 25 ml (A subunit or monomer) yielded an amino terminal sequence of: SEQ ID NO:1

Ala Pro Thr Thr Glu Gly Glu Gln Lys Ala His Glu Val Val

which is identical to the A chain monomers of VEGF AA, Conn et al., Proc. Natl. Acad. Sci. USA 87: 2628-2632 (1990). The peak of absorbance eluting at approximately 23 ml (B subunit or monomer) yielded an N-terminal sequence of: SEQ ID NO:2

Ala Leu Ser Ala Gly Asn Xaa Ser Thr Ser Thr Glu Met Glu Val Val
Pro Phe Asn Glu Val

plus a nearly equal amount of a truncated form of the same sequence missing the first three amino acid residues. The missing Xxx residue corresponds to an Asn residue in the cloned cDNA, see below. Since this missing Asn occurs in a classical Asn Xxx Ser/Thr N-glycosylation sequence it is presumed to be glycosylated. The A subunit and the total of both B subunits are recovered in nearly equal amounts supporting the interpretation that the two peptides combine to form an AB heterodimer in VEGF AB.

A sample of the A monomer was treated with either the protease trypsin which cleaves polypeptides on the C-terminal side of lysine and arginine residues or Lys C which cleaves polypeptides on the C-terminal side of lysine by procedures well known in the art. The peptides are isolated by reversed phase - HPLC(RP-HPLC). The amino acid sequences of the isolated peptides are determined using the Edman degradation in the ABI gas phase sequenator in conjunction with the ABI 120 A on line phenylthiohydantoin analyzer following manufacturer's instructions. The amino acid sequences are shown in Figure 1.

Reduced and carboxymethylated A monomer is dried and solubilized in about 0.7 M Tris, about pH 7.8, about 6 M guanidinium chloride containing about 0.1% EDTA. V8 protease is added in 0. 1 M ammonium bicarbonate buffer, about pH 8.0, and the mixture is incubated for about 48 hr at about 37°C. The protease cleaves predominantly on the carboxyl terminal side of glutamic acid residues. The resulting polypeptides were resolved by C_{18} RP-HPLC as above.

The reduced and carboxymethylated A subunit protein solution is adjusted to a pH of about 6.8 with 6 N HCl and dithiotreitol is added to a final concentration of 2 M for reduction of any methionine sulfoxide to methionine residues. After about 20 hr of reduction at about 39°C the protein is repurified by C_4 HPLC. The product is dried and cleaved on the carboxyl terminal side of methionine residues by 200 µl of 40 mM cyanogen bromide in about 70 % (v/v) formic acid under an argon atmosphere at about 20°C for about 24 hr in the dark. The cleavage products are resolved by C_{18} RP-HPLC. The amino acid sequence is shown in Figure 1, see Conn et al., Proc. Natl. Acad. Sci USA 87:2628-2632 (1990).

The full length 190 amino acid residue protein translation product of the VEGF AB, A monomer or subunit, which is now known to be identical with the VEGF AA, A monomer, and its cDNA coding sequence are shown in Figures 2 and 6. The mature amino terminus begins at residue 27, immediately following a typical hydrophobic secretory leader sequence. A single potential N-glycosylation site exists at Asn_{100}. Most (143 amino acid residues) of the 164 residues of the reduced and carboxymethylated mature subunit including the amino terminus and HPLC reversed phase-purified products of tryptic (T), Lys-C (L), Staphylococcus aureus V8 protease (V8) and cyanogen bromide (CB) cleavages, were determined by direct microsequencing (Applied Biosystems 470A) using a total of 5 µg of protein. All residues identified by amino acid sequencing are denoted by arrows pointing to the right either directly beneath the mature processed sequence following the bracket at residue 27 for the amino terminal determination of the whole subunit or, for residues identified from the polypeptide cleavage products, above the double-headed arrows spanning the length of the particular polypeptide. One listed pair of polypeptides, V18A and V18B, was sequenced as a mixture and, therefore, are only confirmatory of the cDNA-deduced amino acid sequence, see Figures 1 and 5.

Samples of the reduced and carboxymethylated pure VEGF AB, A and B monomers, were each digested

with the Lys-C endoproteinase, which cleaves polypeptides on the C-terminal side of lysine residues. The peptides were isolated by reverse phase HPLC and their amino acid sequences were determined as described above. The locations of the peptides in the final VEGF AB, A and B sequences are shown in Figure 2 and Figure 3, respectively.

The full length coding region of the A subunit or monomer is determined from three sets of overlapping cDNA clones. Degenerate oligonucleotide primers based on the amino acid sequences Phe-Met-Asp-Val-Tyr-Gln from polypeptide L42 (residues 42-47) and Cys-Lys-Asn-Thr-Asp from polypeptide T38 (residues 164-168) (see Figure 1) were used to PCR amplify the central region of the cDNA for VEGF A chain following the procedure of Saiki et al., Science 230: 1350- 1354 (1985). A single band migrating at 420 bp was gel purified, digested with SalI, ligated into pGEM3Zf(+) and sequenced. The nucleotide sequence obtained (p4238) was used to design antisense and sense PCR primers to amplify the 5' and 3' ends of the cDNA according to the protocol described by Frohman et al. Proc. Natl. Acad. Sci. USA 85:8998-9002 (1988). These 5' and 3' clones are denoted p5-15 and pW3, respectively. Regions of complete DNA sequences, excluding the primers, determined for each set of clones are indicated by double-headed arrows above the nucleotide sequence. In addition to the cDNA coding the 164 amino acid secreted form identified by protein sequencing, two alternatively spliced cDNAs encoding a 146 amino acid and a 214 amino acid forms are cloned and sequenced, Figures 4, 5 and 6.

The full length coding region of the B subunit or monomer is determined from four sets of overlapping cDNA clones. Degenerate oligonucleotide primers based on the amino acid sequences from polypeptide L50 are used to PCR amplify the central region of the cDNA for VEGF AB, B monomer, following the procedure of Saiki et al., Science 230: 1350-1354 (1985). A single band migrating at 108 bp was gel purified, digested with SalI, ligated into pGEM3Zf(+) and sequenced. The nucleotide sequence obtained (pYG) was used to design antisense and sense PCR primers to amplify the 5' and 3' ends of the cDNA according to the protocol described by Frohman et al. Proc. Natl. Acad. Sci. USA 85:8998-9002 (1988). These 5' and 3' clones are denoted p5V2 and p3V2, respectively. Additional 5' end sequences are determined from clone 202 isolated from a cDNA library prepared from GS-9L poly A$^+$ RNA. Regions of complete DNA sequences, excluding the primers, determined for each set of clones are indicated by double-headed arrows above the nucleotide sequence. The entire base sequence for the 158 amino acid microheterogeneous B subunit and the 138 amino acid microheterogeneous B subunit are shown in Figures 7 and 8.

The full length coding region of the C subunit or monomer is determined from three sets of overlapping cDNA clones. Degenerate oligonucleotide primers based on the amino acid sequence Phe Ser Pro Ser Cys Val and Glu Met Thr Phe Ser Gly from rat VEGF B subunit are used to PCR amplify the central region of the cDNA of VEGF C chain following the procedure of Saiki et al., Science 230: 1350-1354 (1985). A band migrating at 180 bp is gel purified, reamplified and digested with SalI, ligated into pGEM3Zf(+) and sequenced. The nucleotide sequence obtained (pFSEM') is used to design antisense and sense PCR primers to amplify the 5' and 3' ends of the cDNA according to the protocol described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). The 5' and 3' clones are denoted p5:16 and p3:19, respectively. The entire base sequence and amino acid sequence for the C subunit are shown in Figure 9.

It is intended that vascular endothelial cell growth factor of the present invention exist as a heterodimer consisting of an A microheterogeneous and/or alternatively spliced subunit or a B microheterogeneous and/or alternatively spliced subunit combined with a C microheterogeneous and/or alternatively spliced subunit. It is further intended that VEGF homodimer of the present invention exist as two C subunits. The native forms of the A, B, C subunits may be processed form alternatively spliced full length translation products. The heterodimers or heterodimeric species can be depicted as: A+B, A+C or B+C with the A, B or C subunits existing in any of the alternatively spliced or microheterogeneous forms. The homodimers or homodimeric species can be formed by combinations of any of the alternatively spliced or microheterogeneous forms. It is also intended that the invention include all of the individual subunit forms of the A subunit, the B subunit and the C subunit of VEGF.

It is further intended that the nucleotide sequence for vascular endothelial cell growth factor be interpreted to include all codons that code for the appropriate amino acids in the sequence for each of the vascular endothelial growth factor subunits, as indicated by the degeneracy of the genetic code. It is further intended that the nucleotide sequence and the amino acid sequence for VEGF subunits include truncated genes or proteins that result in proteins which exhibits biological activity similar to vascular endothelial cell growth factor. The scope of the invention is intended to include all naturally occwring mutations and allelic varients and any randomly generated artifical mutants which may change the sequences but do not alter biological activity as determined by the ability to stimulate the division of vascular endothelial cells.

The above described heterodimers, homodimers and subunits of vascular endothelial cell growth factor are characterized by being the products of chemical synthetic procedures or of procaryotic or eucaryotic host

expression of the DNA sequences as described herein. A monomer is defined as a subunit that is not incorporated in an oligomeric unit. Expression of the recombinant VEGF genes (recombinant DNA) is accomplished by a number of different host cells which contain at least one of a number of expression vectors. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of recombinant DNA sequences or genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express genes in a variety of hosts such as bacteria, bluegreen algae, yeast cells, insect cells, plant cells and animal cells, with mammalian cells being preferred. The genes may also be expressed using any of a number of virus expression systems. Specifically designated vectors allow the shuttling of DNA between bacteria-yeast, bacteria-plant or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selective markers, a limited number of useful restriction enzyme sites, a high copy number, strong promoters and efficient translational stop signals. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and to initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses and cosmids. The expression of mammalian genes in cultured mammalian cells is well known in the art. Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Edition, Book 3, Cold Springs Harbor Laboratory Press (1989) and Cwrent Protocols In Molecular Biology, Ausubel et. al. Eds. Greene Publishing Associates and Wiley-Interscience, 1987 and supplements, disclose various mammalian expression vectors and vector systems along with methods for the introduction of recombinant vectors into mammalian cells. The cDNA for the monomemc forms of the A, B and C subunits can be expressed in a system such as that described by Linemeyer et al., European Patent Application, Publication No. 259,953. The cDNA is incorporated into a commercially available plasmid such as pKK 223-3 (Pharmacia) as modified as by Linemeyer et al. and expressed in E. coli. Other expression systems and host cells are well known in the art.

The high Cys content and glycoslyation sites of the A, B and C subunits along with the structure of the homo- and heterodimers suggest that expression of biologically active proteins can be carried out in animal cells. Expression may be carried out in Chinese hamster ovary (CHO) cells with the cloned VEGF DNA cotransfected with the gene encoding dihydrofolate reductase (dhfr) into dhfr- CHO cells, see Sambrook et al. Transformants expressing dhfr are selected on media lacking nucleosides and are exposed to increasing concentrations of methotrexate. The dhfr and VEGF genes are thus coamplified leading to a stable cell line capable of expressing high levels of VEGF. The plasmid is designed to encode either an A subunit, a B subunit or a C subunit or a combination of any two of these subunits. The two cDNAs are operably attached so that the protein produced will be dimeric and will have VEGF biological activity. Operably attached refers to an appropriate sequential arrangement of nucleotide segments, cDNA segments or genes such that the desired protein will be produced by cells containing an expression vector containing the operably attached genes, cDNA segments or nucleotides. Plasmids containing a single subunit species may be used to cotransfect a suitable cell line.

The expressed proteins (homodimers or heterodimers) are isolated and pwified by standard protein purification processes. It is to be understood that the expression vectors capable of expressing heterodimeric forms of VEGF will contain two DNA sequences which will encode either an A subunit and/or a DNA sequence which will encode a B subunit and/or a DNA sequence which will encode a C subunit. Expression vectors capable of expressing homodimeric forms of VEGF will contain either one or two DNA sequences which encode either two A, two B or two C subunits.

The ability of the various species of VEGF to stimulate the division of vascular endothelial cells makes this protein in all microheterogeneous forms and alternative splicing forms useful as a pharmaceutical agent. The protein as used herein is intended to include all microheterogeneous forms as previously described. The protein can be used to treat wounds of mammals including humans by the administration of the novel protein to patients in need of such treatment.

The novel method for the stimulation of vascular endothelial cells comprises treating a sample of the desired vascular endothelial cells in a nutrient medium with mammalian VEGF, preferably human or rat, at a concentration of about 1-10 ng/ml. If the vascular endothelial cell growth is conducted in vitro, the process requires the presence of a nutrient mediuin such as DMEM or a modification thereof and a low concentration of calf or bovine serum such as about 0 to 2% by volume. Preservatives such as antibiotics may also be included; these are well known in the art.

The novel growth factors of this invention are useful for the coverage of artificial blood vessels with vascular endothelial cells. Vascular endothelial cells from the patient would be obtained by removal of a small sequent of peripheral blood vessel or capillary-containing tissue and the desired cells would be grown in culture in the presence of VEGF and any other supplemental components that might be required for growth. After growth of adequate numbers of endothelial cells in culture to cover a synthetic polymeric blood vessel the cells would be plated on the inside surface of the vessel, such as fixed umbilical vein, which is then implanted in the patient.

Alternatively, tubular supports are coated in vitro with VEGF prior to implantation into a patient. Following implantation endothelial cells migrate into and grow on the artificial surface. Prior coating of the artificial vessel either covalently or noncovalently, with proteins such as fibrin, collagen, fibronectin or laminin would be performed to enhance attachment of the cells to the artificial surface. The cell-lined artificial vessel would then be surgically implanted into the patient and, being lined with the patients own cells, would be immunologically compatible. The non-thrombogenic endothelial cell lining should decrease the incidence of clot formation on the surface of the artificial vessel and thereby decrease the tendency of vessel blockage or embolism elsewhere.

The novel proteins are also used for the production of artificial vessels. Vascular endothelial cells and smooth muscle cells from the patient would be obtained and grown separately in culture. The endothelial cells would be grown in the presence of VEGF as outlined above. The smooth muscle would be grown in culture by procedures well known in the art. A tubular mesh matrix of a biocompatible polymer (either a synthetic polymer, with or without a coating of proteins, or a non-immunogenic biopolymeric material such as surgical suture thread) would be used to support the culture growth of the smooth muscle cells on the exterior side and vascular endothelial cells on the interior surface. Once the endothelial cells form a confluent monolayer on the inside surface and multiple layers of smooth muscle cells cover the outside, the vessel is implanted into the patient.

The novel peptides can also be used for the induction of tissue repair or growth. The pure VEGF would be used to induce and promote growth of tissue by inducing vascular growth and /or repair. The peptide can be used either topically for tissue repair or intravascularly for vascular repair. For applications involving neovascularization and healing of surface wounds the formulation would be applied directly at a rate of about 10 ng to about 1 mg/cm^2/day. For vascular repair VEGF is given intraveneously at a rate of about 1 ng to about 100 µg/kg/day of body weight. For internal vascular growth, the formulation would be released directly into the region to be neovascularized either from implanted slow release polymeric material or from slow release pumps or repeated injections. The release rate in either case is about 10 ng to about 100 µg/day/cm^3.

For non-topical application the VEGF is administrated in combination with pharamaceutically acceptable carri ers or diluents such as, phosphate buffer, saline, phosphate buffered saline, Ringer's solution, and the like, in a pharamaceutical composition, according to standard pharamaceutical practice. For topical application, various pharmaceutical formulations are useful for the administration of the active compound of this invention. Such formulations include, but are not limited to, the following: ointments such as hydrophilic petrolatum or polyethylene glycol ointment; pastes which may contain poms such as xanthan gum; solutions such as alcoholic or aqueous solutions; gels such as aluminum hydroxide or sodium alginate gels; albumins such as human or animal albumins; collagens such as human or animal collagens; celluloses such as alkyl celluloses, hydroxy alkyl celluloses and alkylhydroxyalkyl celluloses, for example methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; polyoxamers such as Pluronic® Polyols exemplified by Pluronic® F-127; tetronics such as tetronic 1508; and alginates such as sodium alginate.

The following examples illustrate the present invention without, however, limiting the same thereto.

EXAMPLE 1

Preparation of Medium Conditioned By GS-9L Cells

GS-9L cells were grown to confluence in 175 cm^2 tissue culture flasks in Dulbecco's Modified Eagle's Medium/10% newborn calf serum (DMEM/NCS). At confluence the medium was decanted from the flasks, the flasks were washed with calcium and magnesium free phosphate buffered saline (PBS) and the cells were removed by treatment with a 1X solution of trypsin/EDTA (Gibco). The cells (1 x 10^8) were pelleted by centrifugation, resuspended in 1500 ml of DMEM/5% NCS and plated into a ten level (6000 cm^2 surface area) cell factory (NUNC). After 72 hours incubation at 37° C in a 5% CO_2 atmosphere the medium was decanted and the cell factories were washed 3 times with PBS. The cells were refed with 1500 ml of a 1:2 mixture of Ham's F-12/DMEM containing 25 mM Hepes, pH 7.4, 5 µg/ml insulin, 10 µg/ml transferrin and 1.0 mg/ml bovine serum albumin. This medium was changed with fresh F-12/DMEM after 24 hours and collected every 48 hours after that. The conditioned medium was filtered through a Whatman #1 paper to remove cell debris and stored frozen at -20°C.

EXAMPLE 2

Carboxymethyl-Sephadex Chromatography of VEGF AA and VEGF AB

GS-9L conditioned medium, from Example 1, was thawed and brought to pH 6.0 with 1 M HCl. Two grams

of CM Sephadex C-50 cation exchange (Pharmacia) resin preequilibrated in PBS adjusted to pH 6.0 with 1 N HCl were added to 20 liters of conditioned medium. The mixture was stirred at low speed for 24 hours at 4° C. The resin was then allowed to settle and the medium was siphoned off. The remaining resin slurry was packed into a 3.0 cm diameter column and any remaining medium was allowed to drain off. Unbound protein was washed off the column with 0.05 M sodium phosphate, pH 6.0, containing 0.15 M NaCl. Vascular endothelial growth factor activity was eluted from the column with a subsequent wash of 0.05 M sodium phosphate, pH 6.0, containing 0.6 M NaCl.

EXAMPLE 3

Concanavalin A (Con A) Lectin Affinity Chromatography of VEGF AA and VEGF AB

A 0.9 cm diameter column containing about 5 ml of packed Con A agarose (Vector Laboratories) was equilibrated with 0.05 M sodium acetate, pH 6.0, containing 1 mM Ca^{++}, 1 mM Mn^{++} and 0.6 M NaCl. The active eluate from the CM Sephadex C-50 column, Example 2, was applied to the Con A agarose and unbound protein was washed from the column with equilibration buffer. The column was then rinsed with three column volumes of 0.05 M sodium acetate, pH 6.0, containing 1 mM Ca^{++}, 1 mM Mn^{++} and 0.1 M NaCl. Bound protein was subsequently eluted from the column by application of this buffer supplemented with 0.32 M α-methyl mannoside and 0.28 M α-methyl glucoside.

EXAMPLE 4

Polyaspartic Acid WCX HPLC Cation Exchange Chromatography of VEGF AA and VEGF AB

The active eluate from the Con A column, Example 3, was applied to a 25 cm x 4.6 mm poly(aspartic acid) WCX cation exchange HPLC column (Nest Group) pre-equilibrated in 0.05 M sodium phosphate buffer, pH 6.0. The column was eluted with a linear gradient of 0 to 0.75 M NaCl in this buffer over 60 minutes at a flow rate of 0.75 ml/min collecting 0.75 ml fractions. VEGF AB activity present in fractions eluting between approximately 21.7 and 28.5 ml were pooled.

EXAMPLE 5

Metal Chelate Chromatography

The active fractions eluted from the poly(aspartic acid) WCX column, Example 4, that contain VEGF AB were pooled, adjusted to pH 7.0 and loaded onto a 1 x 10 cm column of Pharmacia Chelating Sepharose 6B charged with an excess of copper chloride and equilibrated in 0.05 M sodium phosphate, pH 7.0, containing 2 M NaCl and 0.5 mM imidazole (A buffer). VEGF AB was eluted from the column with a gradient from 0-20% B over 10 minutes, 20-35% B over 45 minutes and 35-100% B over 5 minutes at a flow rate of 0.3 ml/min, where B buffer was 0.05 M sodium phosphate, pH 7.0, containing 2 M NaCl and 100 mM imidazole. The active fractions containing VEGF AB activity eluting between 12.6 and 22.8 ml of the gradient effluent volume were pooled.

EXAMPLE 6

Reverse Phase Chromatography

The fractions containing VEGF AB activity pooled from the metal chelate column, Example 5 were loaded onto a 4.6 mm x 5 cm Vydac C_4 reverse phase HPLC column (5 μm particle size) equilibrated in solvent A (0.1% trifluoroacetic acid (TFA)). The column was eluted with a gradient of 0-30% solvent B over 15 minutes, 30% B for an additional 15 minutes, then 30-45% B over 22.5 minutes and finally 45-100% B over 5.5 minutes where solvent B = A containing 67% acetonitrile. The flow rate was maintained at 0.75 ml/min. The active VEGF AB fractions eluting between approximately 32.2 and 37.5 ml of the gradient effluent volume were pooled.

EXAMPLE 7

Mitogenic Assays

Human umbilical vein endothelial cells (HUVE) were plated on gelatin-coated 48 well tissue culture dishes

at a density of 5000 cells/well in 500 µl of Medium 199 containing 20% heat inactivated fetal calf serum (FCS). Samples to be assayed were added at the time of plating. The tissue culture plates are incubated at 37° C for 12 hours and 2 microcuries of tritiated thymidine (NEN, 20 Ci/mmol) was added per ml of assay medium (1.0 µCi/vell). The plates were incubated for a further 60 hr, the assay medium was removed and the plates were washed with Hanks balanced salt solution containing 20 mM Hepes, pH 7.5, and 0.5 mg/ml bovine serum albumin. The cells were lysed and the labelled DNA solubilized with 200 µl of a solution containing 2 gm of sodium carbonate and 400 mg sodium hydroxide in 100 ml water. The incorporated radioactivity was determined by liquid scintillation counting.

The concentration of VEGF AB which elicited a half-maximal mitogenic response in HUVE cells was approximately 2 ± 1 ng/ml. The glycosaminoglycan heparin, which is required in these assays at a level of 10-100 µg/ml to promote a response to a positive control, acidic fibroblast growth factor, does not enhance mitogenic stimulation of these cells by VEGF AB.

EXAMPLE 8

Purity And Protein Structural Characterization of VEGF AB

Purity of the protein under non-reducing conditions was determined by SDS-PAGE in 12.5% crosslinked gels according to the method of Laemmli, Nature 227: 680-685 (1970). The silver-stained gel contained a single band with an apparent mass of approximately 58 kDa. VEGF AB migrated in SDS-PAGE under reducing conditions in 15% crosslinked gels as a broad silver-stained band with apparent molecular mass of approximately 23 kDa.

VEGF AB was stored a 4°C in the aqueous trifluoroacetic acid (TFA)/acetonitrile mixture used to elute the homogeneous protein in reversed phase C_4 HPLC chromatography at the final stage of the purification protocol previously described. Aliquots of the purified protein (1-2 µg) were vacuum evaporated to dryness in acid-washed 10 x 75 mm glass tubes and reduced for 2 hours at 50°C in 100 µl of 0. 1 M Tris buffer, pH 9.5, and 6 M guanidinium chloride containing 0.1% EDTA and 20 mM dithiothreitol (Calbiochem, Ultrol grade) under an argon atmosphere. The reduced protein was subsequently carboxymethylated for 1 hour at 20°C by the addition of 100 µl of 0.7 M Tris, pH 7.8, containing 0.1 % EDTA, 6 M guanidinium chloride, 9.2 µM unlabeled iodoacetic acid and 50 µCi of iodo[2-^{14} C]acetic acid (17.9 mCi/mmole, Amersham). After completion of the carboxymethylation, the mixture was loaded directly onto a 4.6 mm x 5.0 cm Vydac C_4 column which had been preequilibrated in 0.1% TFA. The reduced and carboxymethylated protein was repurified by elution with a 45 minute linear gradient of 0 to 67% (v/v) acetonitrile in 0.1% TFA at a flow rate of 0.75 ml/min and stored in this elution solution at 4°C. The reduced and carboxymethylated protein eluted as two peaks at approximately 23 and 25 ml that were of approximately equal area as determined by monitoring absorbance at 210 nm.

Samples of the two protein subunits isolated after reduction and carboxymethylation were each applied to polybrene-coated glass fiber filters and their N-terminal sequences were determined by Edman degradation in an ABI gas phase microsequencer in conjunction with an ABI 120A on line phenylthiohydantoin analyzer following manufacturers instructions. The peak of absorbance eluting at approximately 25 ml (A subunit) yielded an amino terminal sequence Ala Pro Thr Thr Glu Gly Glu Gln Lys Ala His Glu Val Val SEQ ID NO: 1 identical to VEGF AA. The peak of absorbance eluting at approximately 23 ml (B subunit) yielded the N-terminal sequence Ala Leu Ser Ala Gly Asn Xaa Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val SEQ ID NO: 2 plus a nearly equal amount of a truncated form of the same sequence missing the first three residues. The missing X residue corresponds to an Asn in the cloned sequence. Since this missing Asn occurs in a classical Asn-X-Ser/Thr N-glycosylation sequence it is presumed to be glycosylated. The A and sum of the B chain peptides were recovered in nearly equal amounts supporting the interpretation that the two peptides combine to form an AB heterodimer in VEGF II.

Reduced and carboxymethylated A and B subunits (650 ng each) were each dried by vacuum evaporation in acid-washed 10 x 75 mm glass tubes. Lys C protease (50 ng, Boehringer Mannheim), an enzyme that cleaves on the carboxyl terminal side of lysine residues, was added to each tube in 100 µl of 25 mM Tris, pH 8.5, 0.1 % EDTA. The substrate protein subunits were separately digested at 37°C for 8 hours and the resulting polypeptides resolved by reversed phase HPLC chromatography on a 4.6 mm x 25 cm Vydac C_{18} column equilibrated in 0.1% TFA. Polypeptides were fractionated by elution with a 2 hour linear gradient of 0-67% acetonitrile in 0.1% TFA at a flow rate of 0.75 ml/min at 20°C. Individual peaks were manually collected and stored in this elution solution at 4°C.

The amino acid sequences of the isolated peptides were then determined using Edman degradation in an ABI gas phase sequenator in conjunction with the ABI 120 A on line phenylthiohydantoin analyzer (Applied Biosystems Int.). The peptide sequences are shown in the following Figures 2 and 3. The amino acid sequence

of Lys C fragment L20 (Fig. 5) demonstrates that the form of VEGF AB mature A subunit in the heterodimer is the 164 amino acid form. The amino acid sequence of Lys C fragment L26 (Fig. 3) demonstrates that the form of VEGF AB mature B subunit in the heterodimer is the 135 amino acid form derived from the 158 full length amino acid form.

EXAMPLE 9

Cloning and Sequencing of the VEGF A Monomer

PCR Amplification, Cloning and Sequencing of P4238

Two degenerate oligonu leotides were synthesized in order to amplify the cDNA encoding the peptide sequences of VEGF A subunit between LysC fragment L 42 and tryptic fragment T38. These oligonucleotides were:

L42.2 5' TTTGTCGACTT[TC]ATGGA[TC]GT[N]TA[TC]CA 3' SEQ ID NO:3

T383'B

5' CAGAGAATTCGTCGACA[AG]TC[N]GT[AG]TT[TC]TT [AG]CA 3' SEQ ID NO:4

where N=ACGT

Poly A$^+$ RNA was isolated from GS-9L cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows;

1 µg of GS-9L RNA was annealled to 1 µg of adapter primer TA17, 5' GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT 3' SEQ ID NO:5, by incubating in a volume of 10 µl at 70°C for 5 min. followed by cooling to room temperature. To this reaction was added:

3.0 µl	water
2.5 µl	10X buffer (500 mM Tris-HCl pH 8.3, 750 mM KCl, 100 mM $MgCl_2$, 5 mM spermidine)
2.5 µl	100 mM DTT
2.5 µl	10 mM each dATP, dGTP, dCTP, dTTP
0.6 µl	15 units RNasin
2.5 µl	40 mM Na pyrophosphate
1.5 µl	15 units reverse transcriptase

and the reaction was incubated at 42°C for 1 hour, then diluted to 1 ml in 10 mM Tris-HCl I mM EDTA, pH 7.5.

PCR Reactions:

Primary reaction (100 µl)

10 µl	10X buffer from Perkin Elmer Cetus GeneAmp kit
16 µl	1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
2 µl	first strand GS9L cDNA
2 µl	50 pMoles L42.2
2 µl	50 pMoles T383' B
0.5 µl	2.5 units Amplitaq DNA polymerase
67.5 µl	water

Reaction conditions, 40 cycles of 94°C, 1'; 50°C, 2'30"; 72°C, 2'.

Prep scale secondary reaction:

100 µl	10X buffer
160 µl	1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
10 µl	primary PCR reaction
20 µl	500 pMoles L42.2

20 µl 500 pMoles T383'B
5 µl 25 units Amplitaq DNA polymerase
685 µl water
Reaction conditions 94°C, 1'; 55°C, 2'; 72°C, 2'; 30 cycles.

The PCR product was concentrated by Centricon 30 spin columns, purified on a 1% agarose gel, and digested with restriction endonuclease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of pW-3

Based on the sequence obtained from the p4238 clones, two specific PCR primers were synthesized; oligo 307 5' TTTGTCGACTCAGAGCGGAGAAAGC 3' SEQ ID NO:6 and oligo 289 5' TTTGTCGACGAAAATCACTGTGAGC 3' SEQ ID NO:7. These primers were used in combination with oligoA 17 5'GACTCGAGTCGACATCG 3' SEQ ID NO:8 to amplify the cDNA encoding the COOH terminus of VEGF A subunit using the 3' RACE technique described by Frohman et al., PNAS 85: 8998-9002 (1988).

PCR reactions:

Primary reaction 100 µl
10 µl 10X buffer from Perkin Elmer Cetus GeneAmp kit
18 µl 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
0.35 µl first strand GS-9L cDNA
2 µl 50 pMoles oligo 289
0.5 µl 2.5 units Amplitaq DNA polymerase
67.15µl water
Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 2'; 10 cycles then add 50 pMoles A17, then 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 40' followed by 40 cycles 94°C, 1'; 58°C, 2'; 72°C, 2'.

Prep Scale secondary reaction:

60 µl 10X buffer
108 µl 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
24 µl primary PCR reaction
12 µl 300 pMoles oligo 307
12 µl 300 pMoles oligo A17
3 µl 15 units Amplitaq DNA polymerase
381µl water
Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 2'; 30 cycles.

The PCR product was pwified on a 1% agarose gel and digested with restriction endonuclease Sa1I. The Sa1I fragment was then ligated into Sa1I cut pGEM3Zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of p5-15

Based on the sequence of p4238 clones, two specific PCR primers were synthesized; oligo 113 5'TTTGTCGACAACACAGGACGGCTTGAAG 3' SEQ ID NO:9 and oligo 74 5' TTTGTCGACATACTCCTGGAAGATGTCC 3' SEQ ID NO"10.
These primers were used in combination with oligo A17 5' GACTCGAGTCGACATCG 3' SEQ ID NO:8 to amplify the cDNA encoding the amino terminus of VEGF A subunit using the 5' RACE technique described by Frohman et al., supra. Oligo 151 was synthesized in order to specifically prime VEGF A subunit cDNA from GS-9L RNA. Oligo 151 is 5'
CTTCATCATTGCAGCAGC 3' SEQ ID NO:11.

RNA was isolated from GS-9L cells using the Fast Track RNA isolation kit from Invitrogen using the protocol provided. First strand cDNA synthesis was performed as follows;
One µg of GS9L RNA was annealled to 1 µg of oligo 151 by incubating in a volume of 6 µl at 70°C for 5' followed by cooling to room temperature. To this reaction was added:

1.5 µl 10X buffer (500mM Tris-HCl, pH 8.3, 750 mM KCl, 100 mM $MgCl_2$, 5 mM spermidine)
2.5 µl 10 mM DTT
2.5 µl 10 mM each dATP, dGTP, dCTP, dTTP
0.6 µl 25 units RNasin
2.5 µl 40 mM Na pyrophosphate
9.5 µl 20 units diluted reverse transcriptase

The reaction was incubated at 42°C for 1 hour. Excess oligo151 was removed by Centricon 100 spin columns and the 5′ end of the cDNA was tailed by the addition of dATP and terminal transferase. The tailed cDNA was diluted to a final volume of 150 µl in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.

PCR Reactions:

Primary reaction (50 µl)

5 µl 10X buffer from Perkin Elmer Cetus GeneAmp Kit
8 µl 1.25 mM each stock of dATP,dCTP,dGTP, and dTTP
5 µl first strand GS-9L cDNA prime with oligo 151 and tailed
1 µl 25 pMoles oligo 113
1 µl 25 pMoles oligo A17
1 µl 10 pMoles oligo TA17
0.25 µl 1.25 units Amplitq DNA polymersase
28.75 µl water

Reaction conditions; 1 cycle 94°C 1′; 50°C 2′; 72°C 40′ then 40 cycles of 94°C 1′; 50°C 1′30"; 72°C 2′

Prep scale secondary reaction:

60 µl 10X buffer
96 µl 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
6 µl primary PCR reaction
12 µl 300 pMoles oligo74
12 µl 300 pMoles oligo A17
3 µl 15 units Amplitaq DNA polymerase
411 µl water

Reaction conditions 94°C, 1′; 55°C, 2′; 72°C, 2′ 30 cycles.

The PCR product was concentrated by Centricon 100 spin columns, and digested with restriction endonuclease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method. The base sequence is shown in Fig. 5.

Cloning and sequencing of alternative forms of VEGF A cDNA

Based on the sequence obtained from the p5-15 and pW-3 clones, two specific PCR primers were synthesized; oligo 5′ C 5′ TTTGTCGACAACCATGAACTTTCTGC 3′ SEQ ID NO:12 and oligo 181 5′ TTTGTCGACGGTGAGAGGTCTAGTTC 3′ SEQ ID NO:13. These primers were used together to amplify multiple cDNAs encoding alternative forms of the VEGF A subunit.

Preparative PCR Reaction:

50 µl 10X buffer
80 µl 1.25mM each stock of dATP, dCTP, dGTP, and dTTP
10 µl first strand GS-9L cDNA
10 µl 300pMoles oligo 5′C
10 µl 300pMoles oligo 181
2.5 µl 15 units Amplitaq DNA polymerase
337.5 µl water

Reaction conditions 94°C, 1′; 58°C, 2′; 72°C, 3′; 40 cycles.

The PCR product was extracted with phenol/chloroform, concentrated by Centricon 30 spin columns, precipitated by ethanol, and digested with restriction endonuclease Sal I, and ligated into SalI cut pGEM3Zf(+). The ligation mix was used to transform E.coli XL-1 blue. Plasmid DNA was isolated from white transformants

and sequenced by the dideoxy chain termination method. Three sets of clones were identified. Clone#12 encoded the 190 amino acid form of VEGF A subunit identical to that shown in Fig. 1. The 164 amino acid secreted form of VEGF A subunit is that amino acid sequence running continuously from Ala^{27} to Arg^{190}. Clone#14 has a 135 base pair deletion between the second base of the Asn^{140} codon and the third base of the Arg^{184} codon. This clone thus encodes a 146 aa form of the VEGF A subunit with the conversion of Asn^{140} to Lys^{140}. The 120 amino acid secreted form of VEGF A subunit runs from Ala^{27} to Asn^{140}, which becomes Lys^{140} and does not begin until Cys^{185}, this form also finishes at Arg^{190}, Figure 4. Clone #16 has a 72 base pair insertion between the second and third base of the Asn^{140} codon. This clone thus encodes the 214 amino acid form of the VEGF A subunit with the conversion of Asn^{140} to Lys^{140}, Figure 6.

EXAMPLE 10

Cloning and Sequencing of the VEGF B Subunit

PCR Amplification, Cloning and Sequencing of pYG

Two degenerate oligonucleotides were synthesized in order to amplify the cDNA encoding the peptide sequences of VEGF B on Lys C fragment L50. These oligonucleotides were:

YI 5′ TTTGTCGACATA[TC]AT[TCA]GC[N]GA[TC]GA[AG]C 3′ SEQ ID NO:14

GC 5′ TTTGTCGACTC[AG]TC[AG]TT[AG]CA[AG]CA[N]CC 3′ SEQ ID NO:15 where N=ACGT

RNA was isolated from GS-9L cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows;

1 µg of GS-9L poly A+RNA was annealled to 1 µg of adapter primer TA17,
5′GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT 3′ SEQ ID NO: 5, by incubating in a volume of 10 µl at 70°c for 5 min. followed by cooling to room temperature. To this reaction was added:

3.0 µl	water
2.5 µl	10X buffer (500 mM Tris-Hcl, pH 8.3, 750 mM KCl, 100 mM $MgCl_2$, 5mM spermidine)
2.5 µl	100 mM DTT
2.5 µl	10 mM each dATP, dGTP, dCTP, dTTP
0.6 µl	15 units RNasin
2.5 µl	40 mM Na pyrophosphate
1.5 µl	15 units reverse transcriptase

and the reaction was incubated at 42°C for 1 hour, then diluted to 1 ml in 10 mM Tris-HCl, I mM EDTA, pH 7.5.

PCR Reactions:

Primary reaction (50µl)

5 µl	10X buffer from Perkin Elmer Cetus GeneAmp kit
8 µl	1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
1 µl	first strand GS-9L cDNA
1 µl	50 pMoles oligo YI
1 µl	50 pMoles oligo GC
0.25 µl	1.25 units Amplitaq DNA polymerase
33.75 µl	water

Reaction conditions, 40 cycles of 94°C, 1′; 50°C, 2′; 72°C, 2′.

Prep scale reaction:

60 µl	10X buffer
96 µl	1.25mM each stock of dATP, dCTP, dGTP, and dTTP
12 µl	first strand 659L cDNA
12 µl	500pMoles oligo YI
12 µl	500pMoles oligo GC
3 µl	15 units Amplitaq DNA polymerase
405 µl	water

Reaction conditions 94°C, 1′; 50°C, 2′; 72°C, 2′ 40 cycles.

The PCR product was concentrated by Centricon 30 spin columns and digested with restriction endonuc-

lease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of p3V2

Based on the sequence obtained from the pYG clones, a specific PCR primer was synthesized; oligo HP 5' TTTGTCGACACACCCTAATGAAGTGTC 3' SEQ ID NO:16.
This primer was used in combination with oligo A17 5' GACTCGAGTCGACATCG 3' SEQ ID NO:8 to amplify the cDNA encoding the COOH terminus of the VEGF B subunit using the 3' RACE technique described by Frohman et al., PNAS 85: 8998-9002 (1988).

Preparative PCR reaction:

60 µl	10X buffer from Perkin Elmer Cetus Gene Amp Kit
12 µl	first strand 659L cDNA
96 µl	1.25 mM each of dATP, dCTP, dGTP, dTTP
12 µl	300 pMoles oligo A17
12 µl	300 pMoles oligo HP
3 µl	15units Amplitaq DNA polymerase
405 µl	water

Reaction conditions 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 2'; followed by 40 cycles 94°C, 1', 58°C, 2' and 72°C, 2'.

The PCR product was concentrated by Centricon 30 spin columns, precipitated with ethanol and digested with restriction endonuclease Sa1I. The Sa1I fragment was then ligated into Sa1I cut pGEM3Zf(+). The ligation mix was used to transform E. coli. XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of p5V2

Based on the sequence of pYG clones, two specific PcR primers were synthesized; oligoVL' 5' TTTGTCGACAACAGCGACTCAGAAGG 3' SEQ ID NO: 17 and oligoVS' 5' TTTGTCGACACTGAATATATGAGACAC 3' SEQ ID NO:18. These primers were used in combination with oligo A17 5' GACTCGAGTCGACATCG 3' SEQ ID NO:8 to amplify the cDNA encoding the amino terminus of the VEGF B subunit using the 5' RACE technique described by Frohman et al., supra. Oligo 151 was synthesized in order to prime cDNA from GS-9L RNA Oligo 151 is 5' CTTCATCATTGCAGCAGC 3' SEQ ID NO:11.

Poly A+RNA was isolated from GS9L cells using the Fast Track RNA isolation kit from Invitrogen using the protocol provided. First strand cDNA synthesis was performed as follows:

One µg of GS9L RNA was annealled to 1 µg of oligo 151 by incubating in a volume of 6 µl at 70°C for 5' followed by cooling to room temperature. To this reaction was added:

1.5 µl	10X buffer (500 mM Tris-HCl, pH 8.3, 750 mM KCl, 100 mM $MgCl_2$, 5mM spermidine)
2.5 µl	10 mM DTT
2.5 µl	10 mM each dATP, dGTP, dCTP, dTTP
0.6 µl	25 units RNasin
2.5 µl	40 mM Na pyrophosphate
9.5 µl	20 units diluted reverse transcriptase

The reaction was incubated at 42°c for 1 hour.

Excess oligo 151 was removed by Centricon 100 spin columns and the 5' end of the cDNA was tailed by the addition of dATP and terminal transferase. The tailed cDNA was diluted to a final volume of 150 µl in 10 mM Tris-HCl, 1 mM EDTA,pH 7.5

PCR Reactions:

Primary reaction (50 µl)

5 µl	10X buffer from Perkin Elmer Cetus GeneAmp Kit
8 µl	1.25 mM each stock of dATP,dCTP,dGTP, and dTTP
5 µl	first strand GS9L cDNA primed with oligo151 and tailed

1 µl	25 pMoles oligo VL'
1 µl	25 pMoles oligo A17
1 µl	10 pMoles oligo TA17
0.25 µl	1.25 units Amplitq DNA polymersase
28.75 µl	water

Reaction conditions; 1 cycle 94°C,1'; 58°C, 2'; 72°C, 40' then 40 cycles of 94°C, 1'; 58°C, 2'; 72°C, 2'.

Prep scale secondary reaction

100 µl	10X buffer
160 µl	1.25 mM each stock of dATP, dCTP, dGTP, and dTTP
10 µl	primary PCR reaction
20 µl	500 pMoles oligo VS'
20 µl	300 pMoles oligo A17
5 µl	25 units Amplitaq DNA polymerase
685 µl	water

Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 2' 30 cycles.

The PCR product was extracted with phenol/chloroform, concentrated by Centricon 30 spin columns, precipitated by ethanol, and digested with restriction endonuclease SalI. The SalI fragment was purified on 4% Nu-Sieve Agarose gel then ligated into SalI cut pGEM3Zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of pCV2 and pCV2.1

Based on the sequences of the p3V2 and p5CV2 clones, two specific PCR primers were synthesized; oligo 5'CV2.1
5' TTTGTCGAC[N][N]GCAGGTCCTAGCTG 3' SEQ ID NO;19 and oligo 3'CV2 5'
TTTGTCGAC[N][N]CTAATAAATAGAGGG 3' SEQ ID NO:20.
These primers were used together to amplify the cDNA encoding the VEGF B subunit.

Preparative PCR Reaction:

40 µl	10X buffer
64 µl	1.25 mM each dATP, dTTP, dGTP, dCTP
8 µl	first strand GS-9L cDNA
8 µl	200 pMoles 5'CV2.1
8 µl	200 pMoles 3'CV2
2 µl	10units Amplitaq DNA polymerase
270 µl	water

Reaction conditions: 94°C, 1', 58°C, 2', 72°C, 2'; 40 cycles.

The PCR product was extracted with phenol/chloroform, concentrated by Centricon 30 spin columns, precipitated by ethanol, and digested with restriction endonuclease Sal 1, and ligated into Sal I cut pGEM3Zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated form white transfromants and sequenced by the dideoxy chain termination method. Two sets of clones were identified, one encoded a 158 amino acid sequence and the other encoded a 138 amino acid sequence, see Figures 7 and 8.

cDNA Cloning of VEGF B Subunit

The DNA and protein sequences for the amino terminus of the signal peptide of VEGF B was determined from a cDNA clone isolated from a cDNA library constructed from GS-9L polyA+ RNA.

First Strand Synthesis

Anneal 15.6µl (5ug) GS-9L polyA+ RNA and 2.5µl (2.5ug) oligo dT-XbaI primer by heating to 70° C 5' slow cool to room temperature. Add the following:

5.5µl	10X buffer (500 mM Tris-HCl, pH 8.3 (42° C), 750 mM KCl, 100 mM $MgCl_2$, 5mM spermidine
5.5µl	100mM DTT
5.5µl	10 mM each dATP, dTTP, dCTP, dGTP
1.4µl	(55units) RNasin

5.5µl 40mM NaPPi
13.5µl (55units) reverse transcriptase
Incubate at 42° C 60'.

Second Strand Synthesis:

Assemble reaction mix
50 µl first strand reaction
25 µl 10X buffer (500 mM Tris-HCl, pH7.2, 850 mM KCL, 30 mM $MgCl_2$ 1mg/ml BSA, 100 mM $(NH_4)_2SO_4$
7.5 µl 100 mM DTT
25 µl 1mM NAD
6.5 µl (65units) E. coli DNA Polymerasel
2.5 µl (2.5units) E. coli DNA Ligase
2.5 µl (2 units) E. coli RNase H
135 µl water
Incubate at 14° C for 2h and then incubate 70° C for 10'. Add 1ul (10 units) T4 DNA Polymerase, incubate at 37° C for 10', add 25 µl 0.2M EDTA an extract with phenol/chloroform, then precipitate by the addition of 0.5 volume of 7.5 M ammonium acetate and 3 volumes of ethanol, collect precipitate and resuspend in 20 µl of 10 mM Tris-HCl, pH 7.5, 1mM EDTA.

cDNA Library Construction

The above cDNA was ligated into EcoR1/ Xbal digested LambdaGEM-4 (Promega Biochemicals) after the addition of EcoR1 linkers and digestion with EcoR1 and Xbal. A cDNA library was amplified from ~50, 000 independent clones.

Isolation of Rat VEGF B cDNA Clone

The above cDNA library was screened by placque hybridization using pCV2 as a probe. Hybridization conditions were as follows:

5XSSC (1XSSC is 0.15M sodium chloride, 0.015M sodium citrate,
50% Formamide
5X Denhardt's Solution (1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin)
0.15 mg/ml salmon sperm DNA hybridize overnight at 42° C.

Filters were washed 3 times in 2XSSC, 0.1% SDS at room temerature for 5', then 1 time in 1XSSC, 0.1% SDS at 50C for 30'. Positive clones were identified by autoradiography.

The DNA from phage #202 was digested with restriction endonuclease Spel and the 1.1kb band ligated into Xbal digested pGEM3Zf(+). The ligation mix was used to transform E.coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method. The cDNA sequence and predicted amino acid sequence of the signal peptide are shown in Figures 7 and 8.

The entire nucleotide and amino acid sequence of the 138 amino acid form is shown in Fig. 7. The secreted protein starts at Ala^{24} and continues to Arg^{138}. The entire nucleotide and amino acid sequence of the 158 amino acid form is shown in Figures 8. The secreted protein starts at Ala^{24} and continues to Leu^{158}.

EXAMPLE 11

Cloning and sequencing VEGF C Subunit

PCR Amplification, Cloning and Sequencing of pFSEM'

Two degenerate oligonucleotides were synthesized based on the sequence of rat VEGF B monomer in order to amplify VEGF cDNAs from the human medulloblastoma line TE-671, ATCC HTB (McAllister et al., Int. J. Cancer 20:206-212 [1977]). These oligonucleotides were:

FS 5'TTTGTCGACA TTC AGT CC(N) TC(N) TG(TC) GT 3' SEQ ID NO:21
EM' 5' TTTGTCGACA CTG AGA GAA (N)GT CAT (CT)TC 3'
SEQ ID NO:22
where N= AGCT

Poly A+ RNA was isolated from TE-671 cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows using the cDNA Cycle kit from Invitrogen;

1µl 1µg of TE-671 polyA+ RNA
19µl water

5µl 100mM MeMgOH
6.25µl 0.7M B-mercaptoethanol
2.5µl random primer
2.5µl RNase Inhibitor
10µl 5X RT buffer
2.5µl 25mM dNTPs
1.25µl reverse transcriptase 12.5units

The reaction was incubated for 60' at 42°C, then 3' at 95°C, placed on ice, then an additional 1.25ul reverse transcriptase was added and the reaction incubated an additional 60' at 42°C.

The above procedure was performed in duplicate and the cDNAs pooled to a final volume of 100ul.

PCR Reactions:

Primary reaction (100µl)

10 µl 10X buffer from Perkin Elmer Cetus GeneAmp kit
16 µl 1.25mM each of dATP. dCTP, dGTP, TTP
10 µl first strand TE-671 cDNA
2 µl 50 pmoles FS primer
2 µl 50 pmoles EM' primer
0.5 µl 2.5 units Amplitaq DNA polymerase
59.5 µl water

Reaction conditions: 40 cycles of 90°C, 1'; 2' ramp to 45°C; 2' at 45°C; 2' at 72°C.

Gel Purification

20 µl of the primary PCR reaction was purified on a 4% NuSieve agarose gel. The 180 base pair band was excised from the gel. heated to 65°C for 5' and used directly as template for the secondary PCR reaction.

Secondary PCR reaction 200µl

20 µl 10X buffer from Perkin Elmer Cetus GeneAmp kit
32 µl 1.25mM each of dATP. dCTP, dGTP, TTP
5 µl melted gel slice
4 µl 100 pmoles FS primer
4 µl 100 pmoles EM' primer
1 µl 5 units Amplitaq DNA polymerase
134 µl water

Reaction conditions: 35 cycles of 94°C, 1'; 50°C, 2'; 72°C, 2'

The PCR product was purified on a Qiagen tip 5 column, then digested with restriction endonuclease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+), and the ligation mix used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of p3'.19

Based on the sequence obtained from the pFSEM' clone, a specific PCR primer was synthesized; oligo LH 5' TTTGTCGACA CTG CAC TGT GTG CCG GTG 3' SEQ ID NO:23. This primer was used in combination with oligo A17,5' GACTCGAGTCGACATCG 3' SEQ ID NO:24, to amplify the cDNA encoding the COOH terminus of the VEGF C subunit using the 3' RACE technique described by Frohman et al., PNAS 85:8998-9002 (1988).

Poly A+ RNA was isolated from TE-671 cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows using the cDNA Cycle kit from Invitrogen and the TA17 adapter primer.

TA17 5' GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT 3' SEQ ID NO:5
0.8 µl 1µg of TE-671 polyA+ RNA
20.7 µl water
5 µl 100 mM MeMgOH
6.25 µl 0.7 M B-mercaptoethanol
1.0 µl 0.88 µg primer TA17
2.5 µl RNase Inhibitor
10 µl 5X RT buffer
2.5 µl 25mM dNTPs

1.25 µl reverse transcriptase 12.5 units

The reaction was incubated for 60' at 42°C, then 3' at 95°C, placed on ice, then an additional 1.25ul reverse transcriptase was added and the reaction incubated an additional 60' at 42°C.

3' RACE PCR

20 µl	10 X buffer from Perkin Elmer Cetus GeneAmp kit
32 µl	1.25mM each of dATP. dCTP, dGTP, TTP
20 µl	first strand TE-671 cDNA primed with TA17
2 µl	50 pmoles LH primer
2 µl	50 pmoles A17 primer
1.0 µl	5 units Amplitaq DNA polymerase
123 µl	water

Reaction conditions: 40 cycles of 94C, 1'; 2' at 58°C; 3' at 72°C.

The PCR product was purified on a Qiagen tip 5 column, then digested with restriction endonuclease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+), and the ligation mix used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.

PCR Amplification, Cloning and Sequencing of p5'.16

Based on the sequence obtained from the pFSEM' clone, two specific PCR primers were synthesized; oligo VE' 5' TTTGTCGACA AC ATT GGC CGT CTC CAC C 3' SEQ ID NO:24, and oligo TG' 5' TTTGTCGACA ATC GCC GCA GCA GCC GGT 3' SEQ ID NO:25. These primers were used in combination with oligo A17, 5' GACTCGAGTCGACATCG 3' SEQ ID NO:8, and oligo TA17 5'GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT 3' SEQ ID NO:5 to amplify the cDNA encoding the amino terminus of the VEGF C subunit using the 5' RACE technique described by Frohman et al., PNAS 85: 8998-9002 (1988).

Poly A+ RNA was isolated from TE-671 cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows using the cDNA Cycle kit from Invitrogen and the VE' primer:

1.0 µl	1 µg of TE-671 polyA+ RNA
20.25 µl	water
5 µl	100 mM MeMgOH
6.25 µl	0.7 M B-mercaptoethanol
1.0 µl	1.0 µg primer VE'
2.5 µl	RNase Inhibitor
10 µl	5X RT buffer
2.5 µl	25 mM dNTPs
0.5 µl	AMV reverse transcriptase (Promega) 10units

The reaction was incubated for 60'at 42°C, then 3' at 95°C, placed on ice, then an additional 1.25ul reverse transcriptase was added and the reaction incubated an additional 60' at 42°C. Excess oligo VE' was removed by a Centricon 100 spin column and the 5' end of the cDNA was tailed by the addition of dATP and terminal transferase. The tailed cDNA was diluted to a final volume of 200 ul in 10mM Tris-HCl, 1mM EDTA, pH 7.5.

5' RACE PCR 5 X 100ul

10 µl	10X buffer from Perkin Elmer Cetus GeneAmp kit
16 µl	1.25mM each of dATP. dCTP, dGTP, TTP
10 µl	first strand TE-671 cDNA primed with VE'
2 µl	50 pmoles TG' primer
2 µl	50 pmoles A17 primer
2 µl	20 pmoles TA17 primer
0.5 µl	2.5 units AMplitaq DNA polymerase
57.5 µl	water

Reaction conditions: 40 cycles of 94°C, 1'; 2' ramp to 58°C; 2' at 58°C; 2' at 72°C.

The PCR product was purified on a Qiagen tip 5 column, then digested with restriction endonuclease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+), and the ligation mix used to transform E. coli

XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method. The combined sequences form plasmids pFSEM', p3'19 and p5'16 are shown in Figure 9.

PCR Amplificpotion, Cloning and Sequencing of phVC16 and phVC2

Based on the sequences of the p5'. 16 and p3'. 19 clones, two specific PCR primers were synthesized; oligo 5' GCVB 5' TTTGTCGAC TGG CTC TGG ACG TCT GAG 3' SEQ ID NO:26 and oligo 3'VC 5'TTTGTCGAC ACT GAA GAG TGT GAC GG 3' SEQ ID NO:27. These primers were used together to amplify the cDNA encoding the complete VEGF C subunit.

Poly A+ RNA was isolated from TE-671 cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows using the cDNA Cycle kit from Invitrogen;

0.8µl	1µg of TE-671 polyA+ RNA
19.2µl	water
5µl	100 mM MeMgOH
6.25µl	0.7 M B-mercaptoethanol
2.5µl	oligo dT primer
2.5µl	RNase Inhibitor
10µl	5X RT buffer
2.5µl	25 mM dNTPs
1.25µl	reverse transcriptase 12.5units

The reaction was incubated for 60' at 42C, then 3' at 95C, placed on ice, then an additional 1.25ul reverse transcriptase was added and the reaction incubated an additional 60' at 42C.

PCR Reaction 200 ul

20µl	10X buffer from Perkin Elmer Cetus GeneAmp kit
32µl	1.25mM each of dATP. dCTP, dGTP, TTP
20µl	first strand TE-671 cDNA primed with oligo dT
4µl	50 pmoles 5' GCVB primer
4µl	50 pmoles 3'VC primer
1µl	5 units Amplitaq DNA polymerase
119µl	water

Reaction conditions: 40 cycles of 94°C, 1';; 2' at 50°C; 2'at 72°C.

The PCR product was purified on a Qiagen tip 5 column, then digested with restriction endonuclease SalI. The SalI fragment was then ligated into SalI cut pGEM3Zf(+), and the ligation mix used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method. In the sequences of clones phVC16 and phVC2 base 463 (Fig. 9) was changed from a T to a C eliminating the translational stop codon following amino acid 154; this results in the addition of 16 amino acids following amino acid Lys 154. The nucleotide sequence and the deduced amino acid sequence of this addition is:

```
CAG AGA CCC ACA GAC TGC CAC CTG TGC GGC GAT GCT GTT
Gln Arg Pro Thr Asp Cys His Leu Cys Gly Asp Ala Val
155                 160                 165

CCC CGG AGG TAA
Pro Arg Arg
      170
```

SEQ ID NO:29

In addition clone phVC16 contains a 3 base pair deletion (Figure 9, nucleotide residues 73-75) resulting in the deletion of Gln 25.

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Applicable
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1
Ala Pro Thr Thr Glu Gly Glu Gln Lys Ala His Glu Val
5 10
Val

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Applicable
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
Ala Leu Ser Ala Gly Asn Xaa Ser Thr Glu Met Glu Val
5 10
Val Pro Phe Asn Glu Val
15

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
TTTGTCGACT TYATGGAYGT NTAYCA 26

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
CAGAGAATTC GTCGACARTC NGTRTTYTTR CA 32

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
GACTCGAGTC GACATCGATT TTTTTTTTTT TTTTT 35

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
TTTGTCGACA ACACAGGACG GCTTGAAG 28

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
TTTGTCGACG AAAATCACTG TGAGC 25

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GACTCGAGTC GACATCG 17

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
TTTGTCGACA ACACAGGACG GCTTGAAG 28

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TTTGTCGACA TACTCCTGGA AGATGTCC 28

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
CTTCATCATT GCAGCAGC 18

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
TTTGTCGACA ACCATGAACT TTCTGC 26

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
TTTGTCGACG GTGAGAGGTC TAGTTC 26

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
TTTGTCGACA TAYATHGCNG AYGARC 26

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
TTTGTCGACT CRTCRTTRCA RCANCC 26

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
TTTGTCGACA CACCCTAATG AAGTGTC 27

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
TTTGTCGACA ACAGCGACTC AGAAGG 26

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
TTTGTCGACA CTGAATATAT GAGACAC 27

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
TTTGTCGACN NGCAGGTCCT AGCTG 25

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
TTTGTCGACN NCTAATAAAT AGAGGG 26

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
TTTGTCGACA TTCAGTCCNT CNTGYGT 27

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
TTTGTCGACA CTGAGAGAAN GTCATYTC 28

(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
TTTGTCGACA CTGCACTGTG TGCCGGTG 28

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
TTTGTCGACA ACATTGGCCG TCTCCACC 28

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
TTTGTCGACA ATCGCCGCAG CAGCCGGT 28

(2) INFORMATION FOR SEQ ID NO:26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
TTTGTCGACT GGCTCTGGAC GTCTGAG

(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
TTTGTCGACA CTGAAGAGTG TGACGG 26

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CAG AGA CCC ACA GAC TGC CAC CTG TGC GGC GAT GCT GTT 39

CCC CGG AGG TAA 51

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: amino acids

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gln Arg Pro Thr Asp Cys His Leu Cys Gly Asp Ala Val
5 10

Pro Arg Arg
15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 577 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
AACC                                                                    4

ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG             49
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5                  10                  15

CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA             94
Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
            20                  25                  30

GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC            139
Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
            35                  40                  40

TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC            184
Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
            50                  55                  60

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC            229
Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
            65                  70                  75
```

```
TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
                80                  85                  90

CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
                95                 100                 105

ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
               110                 115                 120

TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
               125                 130                 135

ACA AAG CCA GAA AAT CAC TGT GAG CCT TGT TCA GAG CGG AGA AAG 454
Thr Lys Pro Glu Asn His Cys Glu Pro Cys Ser Glu Arg Arg Lys
               140                 145                 150

CAT TTG TTT GTC CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA 499
His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys
               155                 160                 165

AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA 544
Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu
               170                 175                 180

CGT ACT TGC AGA TGT GAC AAG CCA AGG CGG TGA                 577
Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
               185                 190
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 190 amino acids

(B) TYPE: amino acids

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5                  10                  15

Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
            20                  25                  30

Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
        35                  40                  45

Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
    50                  55                  60

Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
```

65 70 75

Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
80 85 90

Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
95 100 105

Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
110 115 120

Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
125 130 135

Thr Lys Pro Glu Asn His Cys Glu Pro Cys Ser Glu Arg Arg Lys
140 145 150

His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys
155 160 165

Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu
170 175 180

Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
185 190

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 445 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACCA 4

ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG 49
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
5 10 15

CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA 94
Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
20 25 30

GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
35 40 45

TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
50 55 60

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC 229
Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser

```
                    65                  70                  75

TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
                    80                  85                  90

CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
                    95                 100                 105

ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
                   110                 115                 120

TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
                   125                 130                 135

ACA AAG CCA GAA AAA TGT GAC AAG CCA AGG CGG TGA             445
Thr Lys Pro Glu Lys Cys Asp Lys Pro Arg Arg
                   140                 145
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 146 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5                  10                  15

Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
20                  25                  30

Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
35                  40                  45

Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
50                  55                  60

Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
65                  70                  75

Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
80                  85                  90

Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
95                 100                 105

Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
110                115                 120
```

Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
125 130 135

Thr Lys Pro Glu Lys Cys Asp Lys Pro Arg Arg
140 145

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 649 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
                                                                    4
AACC

ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG        49
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5                  10                  15

CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA        94
Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
            20                  25                  30

GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC       139
Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
        35                  40                  45

TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC       184
Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
    50                  55                  60

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC       229
Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
65                  70                  75

TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC       274
Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
            80                  85                  90

CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC       319
Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
        95                  100                 105

ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC       364
Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
    110                 115                 120

TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA       409
Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
125                 130                 135

ACA AAG CCA GAA AAA AAA TCA GTT CGA GGA AAG GGA AAG GGT CAA       454
```

```
Thr Lys Pro Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln
                140                 145                 150

AAA CGA AAG CGC AAG AAA TCC CGG TTT AAA TCC TGG AGC GTT CAC 499
Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His
            155                 160                 165

TGT GAG CCT TGT TCA GAG CGG AGA AAG CAT TTG TTT GTC CAA GAT 544
Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp
        170                 175                 180

CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC 589
Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
    185                 190                 195

AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC 634
Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
200                 205                 210

AAG CCA AGG CGG TGA
Lys Pro Arg Arg                                             649
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 214 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5                  10                  15

Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
            20                  25                  30

Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
        35                  40                  45

Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
    50                  55                  60

Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
65                  70                  75

Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
                80                  85                  90

Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
            95                  100                 105

Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
        110                 115                 120
```

```
Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
                125                 130                 135

Thr Lys Pro Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln
            140                 145                 150

Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His
        155                 160                 165

Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp
    170                 175                 180

Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
185                 190                 195

Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
            200                 205                 210

Lys Pro Arg Arg
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 417 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT  45
Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
  1               5                  10                  15

GGG TTG GCT GTG CAC TCC CAG GGG GCC CTG TCT GCT GGG AAC AAC  90
Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
             20                  25                  30

TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC 135
Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
         35                  40                  45

AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA 180
Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
     50                  55                  60

CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT 225
His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
 65                  70                  75

CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT 270
Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
             80                  85                  90
```

```
GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT 315
Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
 95                 100                 105

CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT 360
Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
 110                 115                 120

CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG 405
Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
 125                 130                 135

GCA GAA AGG TAA
Ala Glu Arg                                              417
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 138 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5                  10                  15

Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
 20                  25                  30

Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
 35                  40                  45

Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
 50                  55                  60

His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
 65                  70                  75

Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
 80                  85                  90

Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
 95                 100                 105

Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
 110                 115                 120

Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
 125                 130                 135

Ala Glu Arg
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 477 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT  45
Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5                  10                  15

GGG TTG GCT GTG CAC TCC CAG GGG GCC CTG TCT GCT GGG AAC AAC  90
Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
            20                  25                  30

TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC 135
Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
        35                  40                  45

AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA 180
Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
    50                  55                  60

CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT 225
His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
65                  70                  75

CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT 270
Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
            80                  85                  90

GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT 315
Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
        95                  100                 105

CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT 360
Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
    110                 115                 120

CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG 405
Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
125                 130                 135

GCA GAA AGG AGG AAA ACC AAG GGG AAG AGG AAG CAA AGC AAA ACC 450
Ala Glu Arg Arg Lys Thr Lys Gly Lys Arg Lys Gln Ser Lys Thr
            140                 145                 150

CCA CAG ACT GAG GAA CCC CAC CTG TGA                         477
Pro Gln Thr Glu Glu Pro His Leu
        155
```

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 158 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5                  10                  15

Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
            20                  25                  30

Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
        35                  40                  45

Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
    50                  55                  60

His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
65                  70                  75

Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
            80                  85                  90

Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
        95                  100                 105

Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
    110                 115                 120

Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
125                 130                 135

Ala Glu Arg Arg Lys Thr Lys Gly Lys Arg Lys Gln Ser Lys Thr
            140                 145                 150

Pro Gln Thr Glu Glu Pro His Leu
        155
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 465 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG GCC  45
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala
 5                  10                  15
```

```
GGG CTG GCG CTG CCT GCT GTG CCC CCC CAG CAG TGG GCC TTG TCT  90
Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser
                20                  25                  30

GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC TTC CAG GAA 135
Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu
                35                  40                  45

GTG TGG GGC CGC AGC TAC TGC CGG GCG CTG GAG AGG CTG GTG GAC 180
Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp
                50                  55                  60

GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG CAC ATG TTC AGC CCA 225
Val Val Ser Glu Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro
                65                  70                  75

TCC TGT GTC TCC CTG CTG CGC TGC ACC GGC TGC TGC GGC GAT GAG 270
Ser Cys Val Ser Leu Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu
                80                  85                  90

AAT CTG CAC TGT GTG CCG GTG GAG ACG GCC AAT GTC ACC ATG CAG 315
Asn Leu His Cys Val Pro Val Glu Thr Ala Asn Val Thr Met Gln
                95                 100                 105

CTC CTA AAG ATC CGT TCT GGG GAC CGG CCC TCC TAC GTG GAG CTG 360
Leu Leu Lys Ile Arg Ser Gly Asp Arg Pro Ser Tyr Val Glu Leu
               110                 115                 120

ACG TTC TCT CAG CAC GTT CGC TGC GAA TGC CGG CCT CTG CGG GAG 405
Thr Phe Ser Gln His Val Arg Cys Glu Cys Arg Pro Leu Arg Glu
               125                 130                 135

AAG ATG AAG CCG GAA AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG 450
Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg
               140                 145                 150

AGG AGA GAG AAG TAG                                        465
Arg Arg Glu Lys
```

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 154 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala
 5                  10                  15

Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser
                20                  25                  30
```

```
Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu
                35                  40                  45

Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp
            50                  55                  60

Val Val Ser Glu Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro
        65                  70                  75

Ser Cys Val Ser Leu Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu
                80                  85                  90

Asn Leu His Cys Val Pro Val Glu Thr Ala Asn Val Thr Met Gln
            95                  100                 105

Leu Leu Lys Ile Arg Ser Gly Asp Arg Pro Ser Tyr Val Glu Leu
        110                 115                 120

Thr Phe Ser Gln His Val Arg Cys Glu Cys Arg Pro Leu Arg Glu
    125                 130                 135

Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg
140                 145                 150

Arg Arg Glu Lys
```

Claims

1. A purified and isolated DNA sequence encoding the C subunit of vascular endothelial cell growth factor.

2. A purified and isolated vascular endothelial cell growth factor C subunit DNA sequence comprising:

```
ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG
GCC GGG CTG GCG CTG CCT GCT GTG CCC CCC CAG CAG TGG GCC
TTG TCT GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC
TTC CAG GAA GTG TGG GGC CGC AGC TAC TGC CGG GCG CTG GAG
AGG CTG GTG GAC GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG
CAC ATG TTC AGC CCA TCC TGT GTC TCC CTG CTG CGC TGC ACC
GGC TGC TGC GGC GAT GAG AAT CTG CAC TGT GTG CCG GTG GAG
ACG GCC AAT GTC ACC ATG CAG CTC CTA AAG ATC CGT TCT GGG
GAC CGG CCC TCC TAC GTG GAG CTG ACG TTC TCT CAG CAC GTT
CGC TGC GAA TGC CGG CCT CTG CGG GAG AAG ATG AAG CCG GAA
AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG AGG AGA GAG AAG
TAG.  SEQ ID NO:40
```

3. Vascular endothelial cell growth growth factor AC DNA comprising an A subunit DNA sequence and a C subunit DNA sequence.

4. Vascular endothelial cell growth growth factor BC DNA comprising a B subunit DNA sequence and a C subunit DNA sequence.

5. A purified and isolated vascular endothelial cell growth factor C subunit DNA sequence comprising:

ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG

GCC GGG CTG GCG CTG CCT GCT GTG CCC CCC CAG CAG TGG GCC
TTG TCT GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC
TTC CAG GAA GTG TGG GGC CGC AGC TAC TGC CGG GCG CTG GAG
AGG CTG GTG GAC GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG
CAC ATG TTC AGC CCA TCC TGT GTC TCC CTG CTG CGC TGC ACC
GGC TGC TGC GGC GAT GAG AAT CTG CAC TGT GTG CCG GTG GAG
ACG GCC AAT GTC ACC ATG CAG CTC CTA AAG ATC CGT TCT GGG
GAC CGG CCC TCC TAC GTG GAG CTG ACG TTC TCT CAG CAC GTT
CGC TGC GAA TGC CGG CCT CTG CGG GAG AAG ATG AAG CCG GAA
AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG AGG AGA GAG AAG
CAG AGA CCC ACA GAC TGC CAC CTG TGC GGC GAT GCT GTT CCC
CGG AGG TAA. SEQ ID NOS:29 & 40

6. Vascular endothelial cell growth factor AC DNA comprising an A subunit DNA sequence selected form the group consisting of: a DNA sequence encoding an 189 amino acid form, a DNA sequence encoding an 165 amino acid form and a DNA sequence encoding a 121 amino acid form, with said A subunit DNA operably attached to a C subunit DNA sequence.

7. Vascular endothelial cell growth factor BC DNA comprising a B subunit DNA sequence selected form the group consisting of a DNA sequence encoding a 135 amino acid form; and a DNA sequence encoding a 115 amino acid form, with said B subunit DNA sequence operably attached to a C subunit DNA sequence.

8. Homodimeric vascular endothelial growth factor DNA comprising C subunit DNA sequences.

9. A vector containing the DNA sequence of any one of claims 3 to 8.

10. A host cell transformed by the vector of claim 9 containing the DNA sequence encoding vascular endothelial cell growth factor.

11. A process for the preparation of vascular endothelial cell growth factor comprising culturing the transformed host cell of claim 10 under conditions suitable for the expression of vascular endothelial cell growth factor and recovering vascular endothelial cell growth factor.

12. Vascular endothelial growth factor made by the process of claim 11.

13. Vascular endothelial cell growth factor AC comprising an A subunit amino acid sequence and a C subunit amino acid sequence.

14. Vascular endothelial cell growth factor BC comprising a B subunit amino acid sequence and a C subunit amino acid sequence.

15. Vascular endothelial cell growth factor CC comprising a C subunit amino acid sequence and a C subunit amino acid sequence.

16. A purified and isolated vascular endothelial cell growth factor C subunit amino acid sequence comprising:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu
Ala Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala
Leu Ser Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro
Phe Gln Glu Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu
Arg Leu Val Asp Val Val Ser Glu Tyr Pro Ser Glu Val Glu
His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg Cys Thr
Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val
Arg Cys Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu
Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys.
SEQ ID NO:41

17. A purified and isolated vascular endothelial cell growth factor C subunit amino acid sequence comprising:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu
Ala Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala
Leu Ser Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro
Phe Gln Glu Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu
Arg Leu Val Asp Val Val Ser Glu Tyr Pro Ser Glu Val Glu
His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg Cys Thr
Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val
Arg Cys Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu
Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys
Gln Arg Pro Thr Asp Cys His Leu Cys Gly Asp Ala Val Pro
Arg Arg. SEQ ID NOS: 29 & 40

18. A tissue repairing pharmaceutical composition comprising a pharmaceutical carrier and an effective tissue repairing amount of the purified vascular endothelial growth factor of any one of claims 13 to 15,

19. The use of the vascular endothelial cell growth factor of any one of claims 13 to 15 for the manufacture of a medicament for promoting tissue repair.

20. The use of the vascular endothelial cell growth factor of any one of claims 13 to 15 for the manufacture of a medicament for stimulating vascular endothelial cell growth.

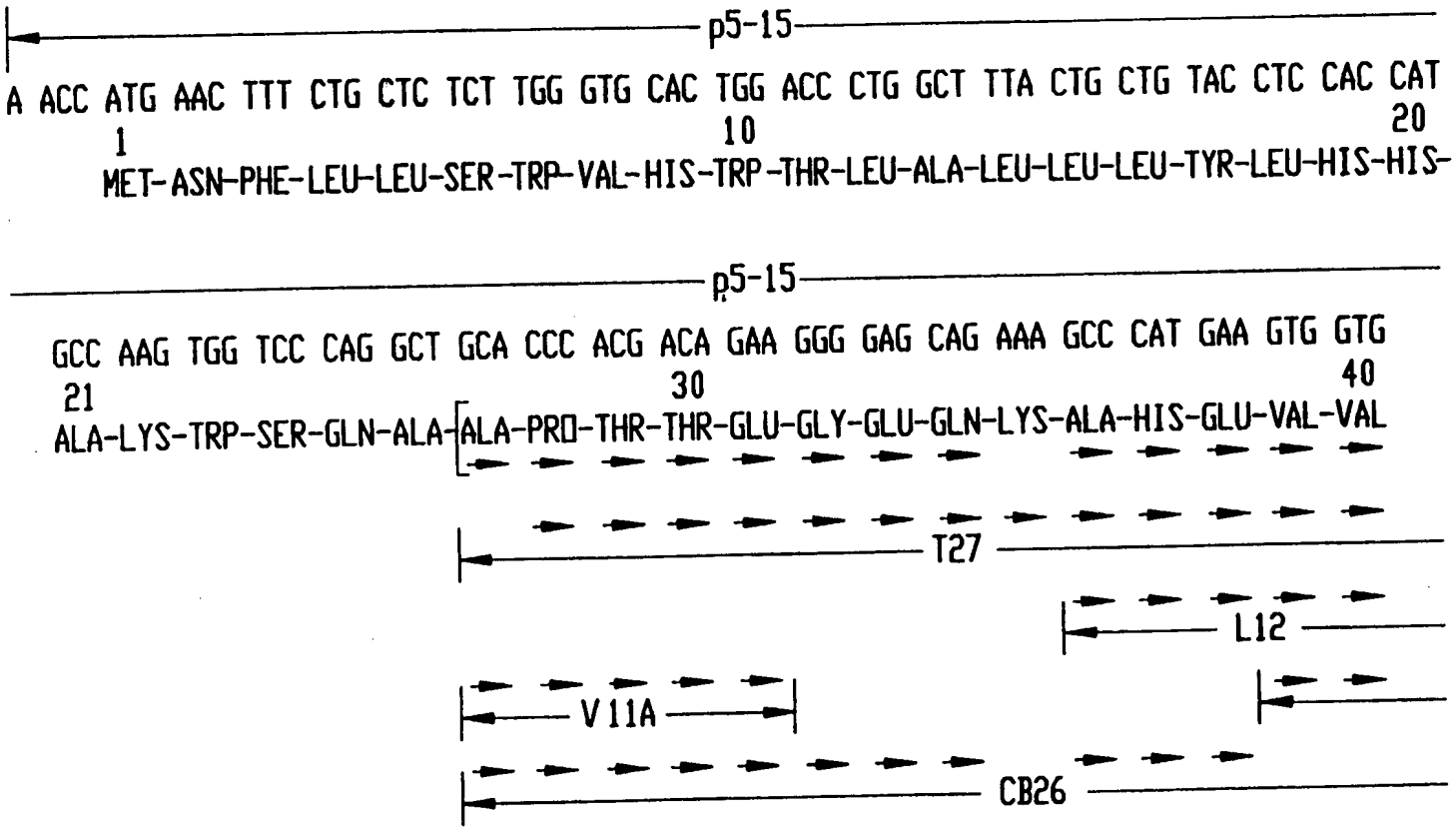

FIG.1A

EP 0 506 477 A1

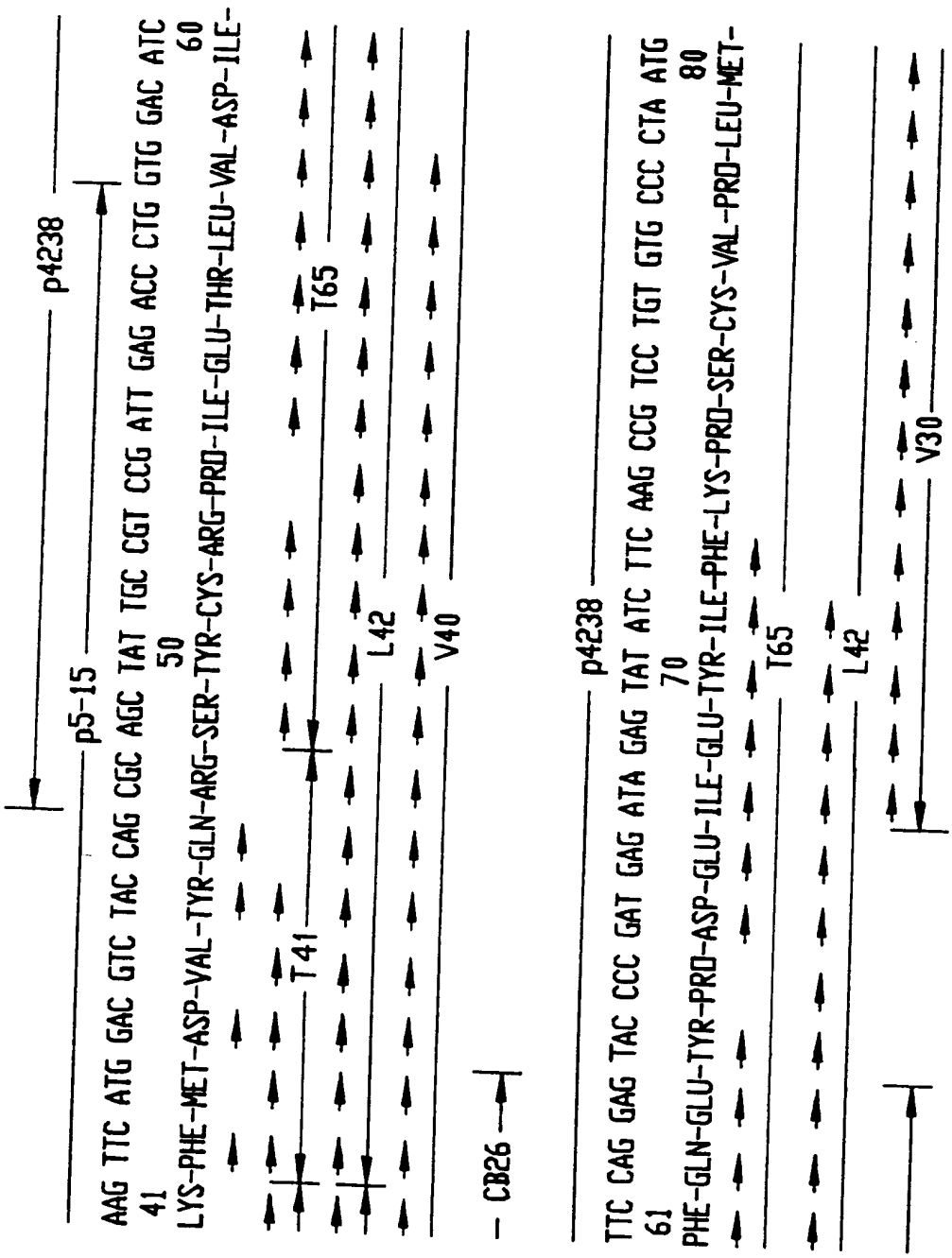
p4238
p5-15
AAG TTC ATG GAC GTC TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC
41 50 60
LYS-PHE-MET-ASP-VAL-TYR-GLN-ARG-SER-TYR-CYS-ARG-PRO-ILE-GLU-THR-LEU-VAL-ASP-ILE-
T41
T65
L42
V40
CB26
p4238
TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC TGT GTG CCC CTA ATG
61 70 80
PHE-GLN-GLU-TYR-PRO-ASP-GLU-ILE-GLU-TYR-ILE-PHE-LYS-PRO-SER-CYS-VAL-PRO-LEU-MET-
T65
L42
V30

FIG.1B

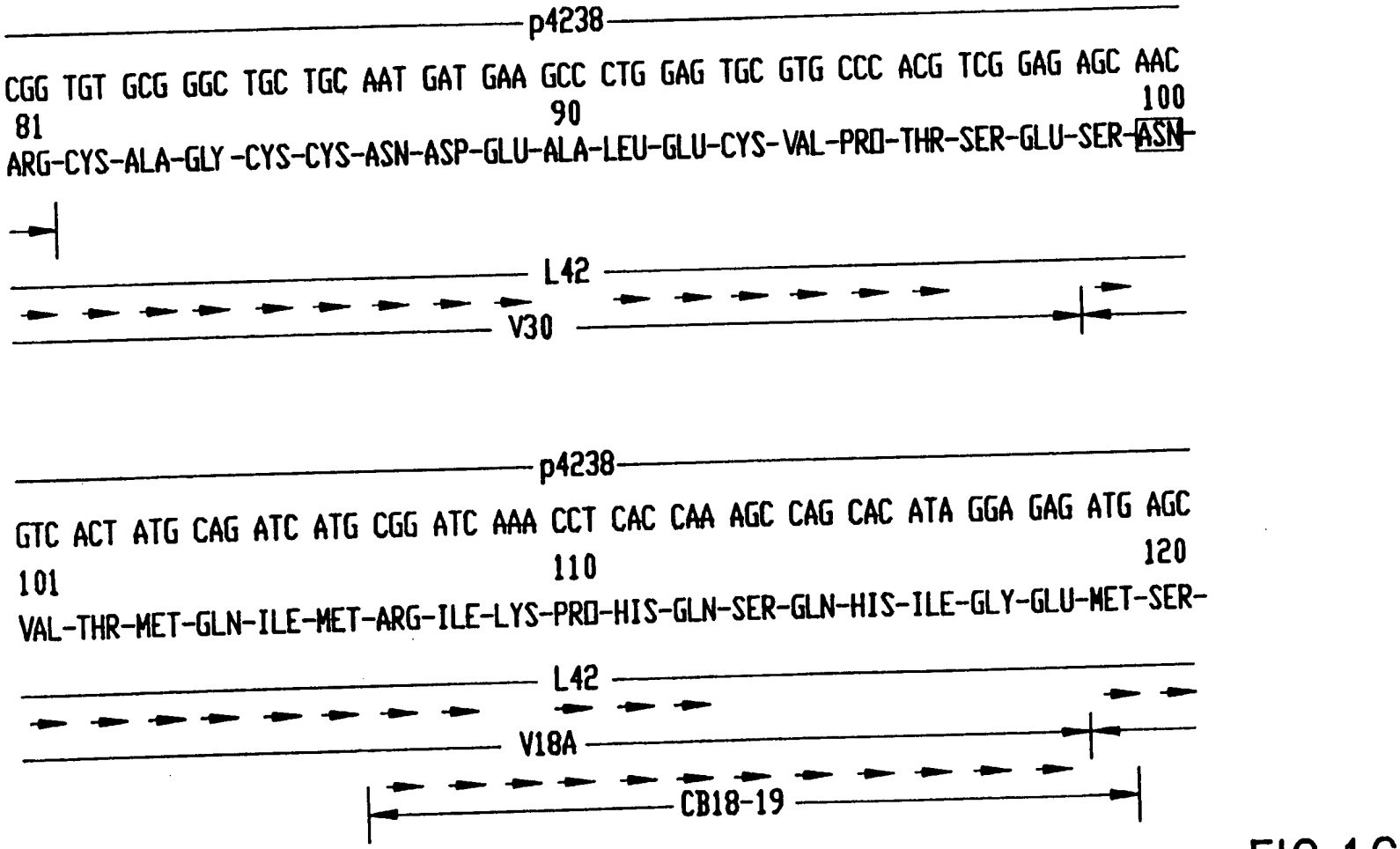

FIG.1C

EP 0 506 477 A1

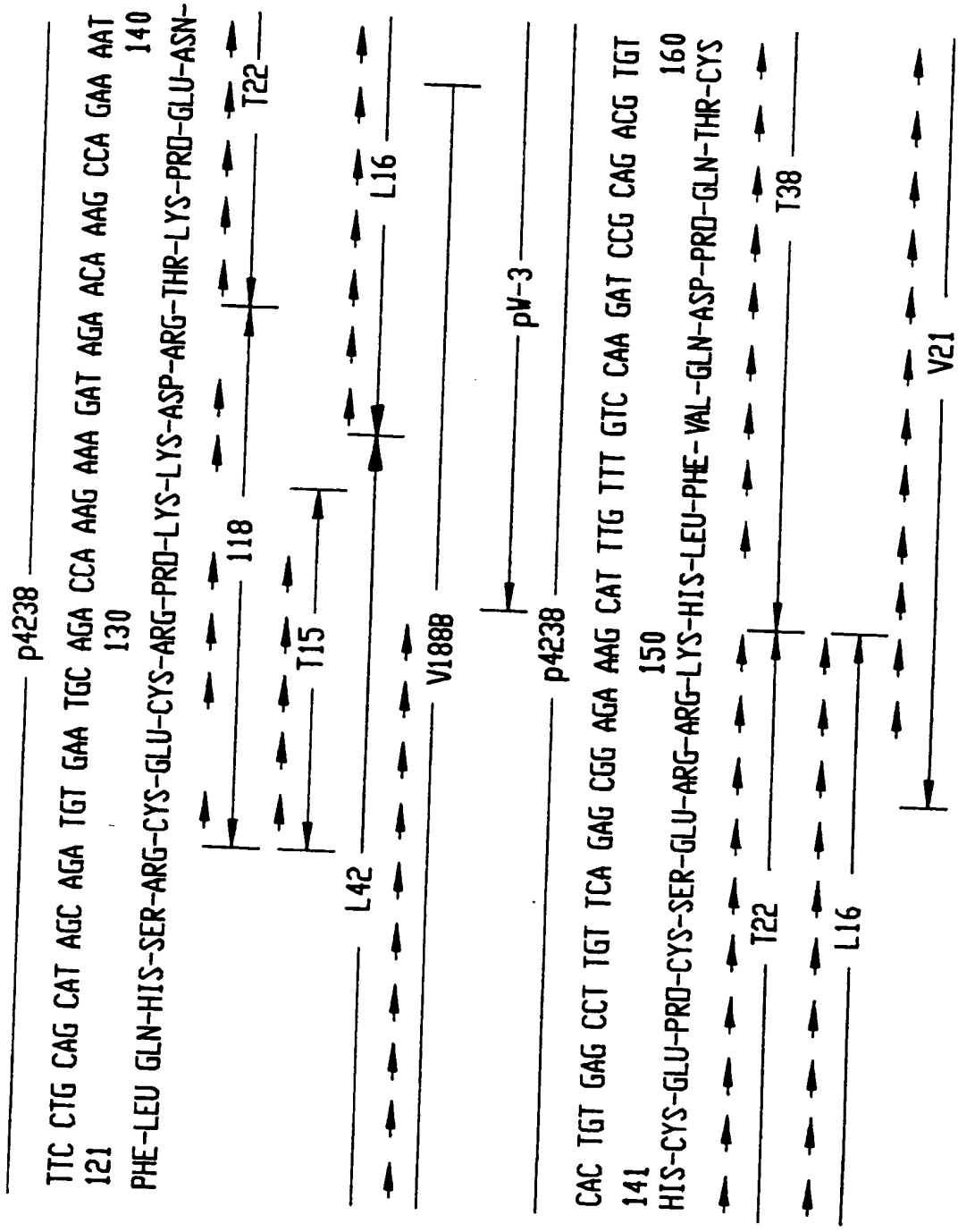
p4238
TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA ACA AAG CCA GAA AAT
121 130 140
PHE-LEU GLN-HIS-SER-ARG-CYS-GLU-CYS-ARG-PRO-LYS-LYS-ASP-ARG-THR-LYS-PRO-GLU-ASN-
118
T22
T15
L42
L16
V1888
pW-3
p4238
CAC TGT GAG CCT TGT TCA GAG CGG AGA AAG CAT TTG TTT GTC CAA GAT CCG CAG ACG TGT
141 150 160
HIS-CYS-GLU-PRO-CYS-SER-GLU-ARG-ARG-LYS-HIS-LEU-PHE-VAL-GLN-ASP-PRO-GLN-THR-CYS
T22
T38
L16
V21

FIG.1D

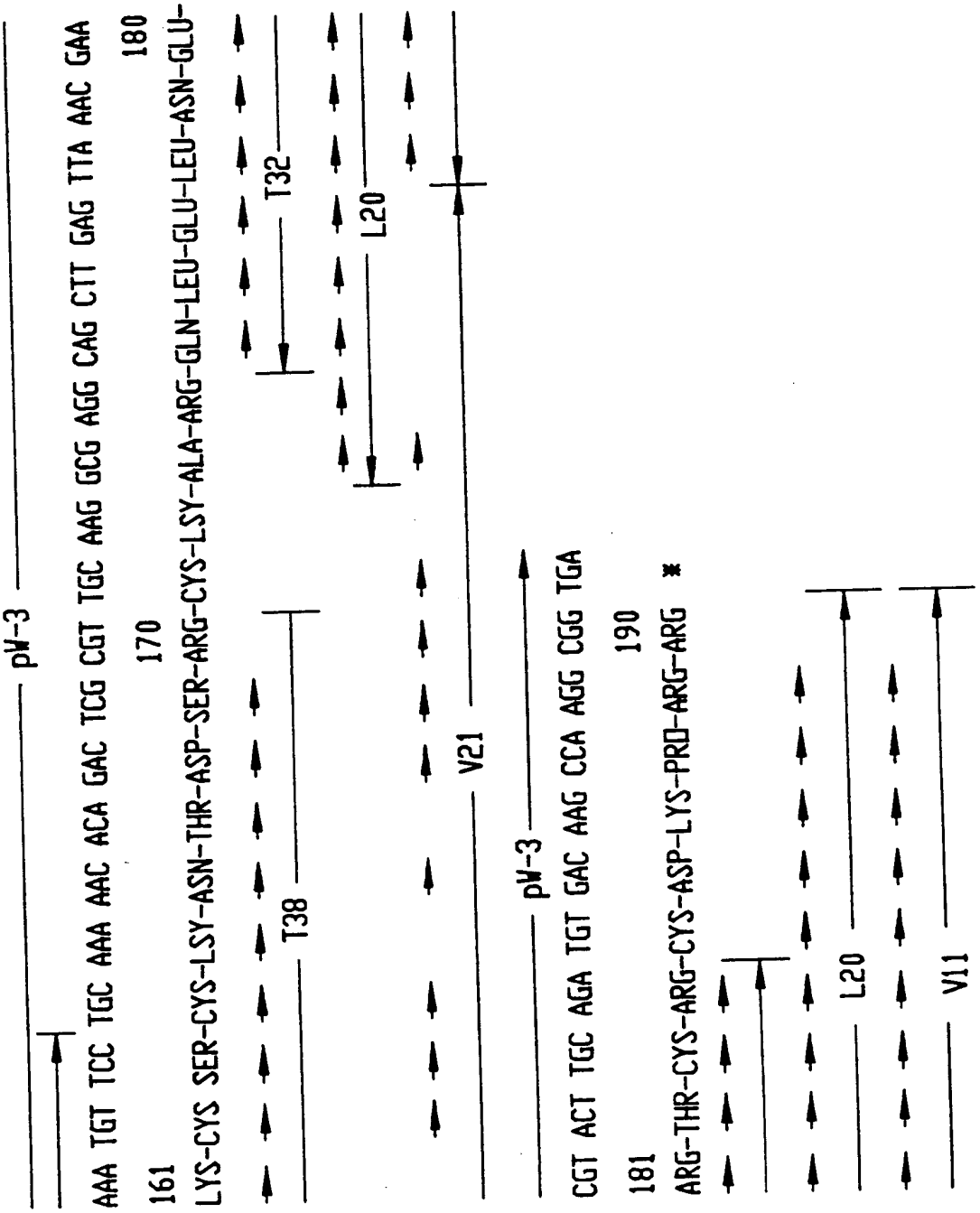
pW-3
AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA
161
170
180
LYS-CYS SER-CYS-LSY-ASN-THR-ASP-SER-ARG-CYS-LSY-ALA-ARG-GLN-LEU-GLU-LEU-ASN-GLU-
T32
T38
L20
V21
pW-3
CGT ACT TGC AGA TGT GAC AAG CCA AGG CGG TGA
181
190
ARG-THR-CYS-ARG-CYS-ASP-LYS-PRO-ARG-ARG *
L20
V11

FIG.1E

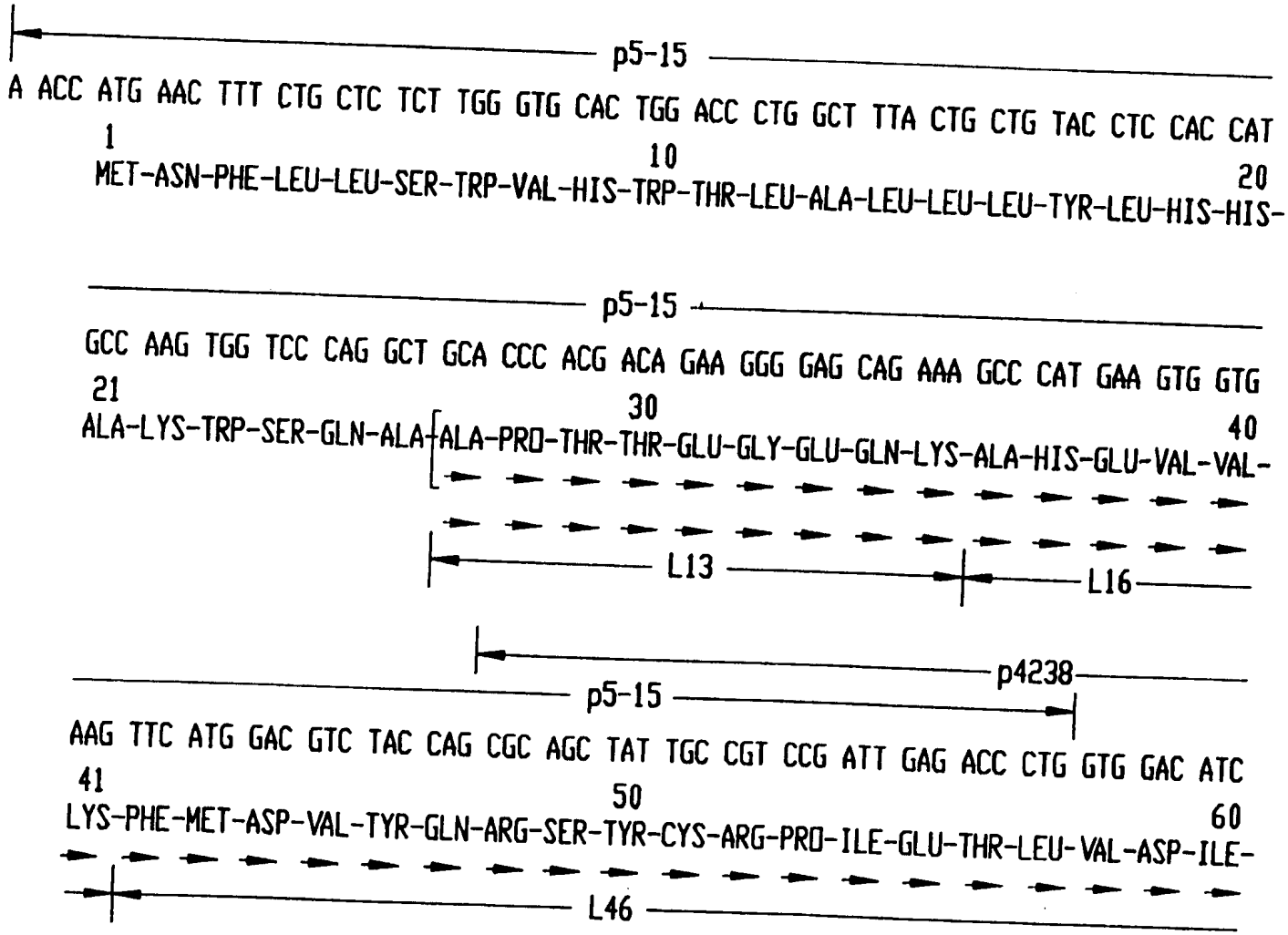

FIG.2A

EP 0 506 477 A1

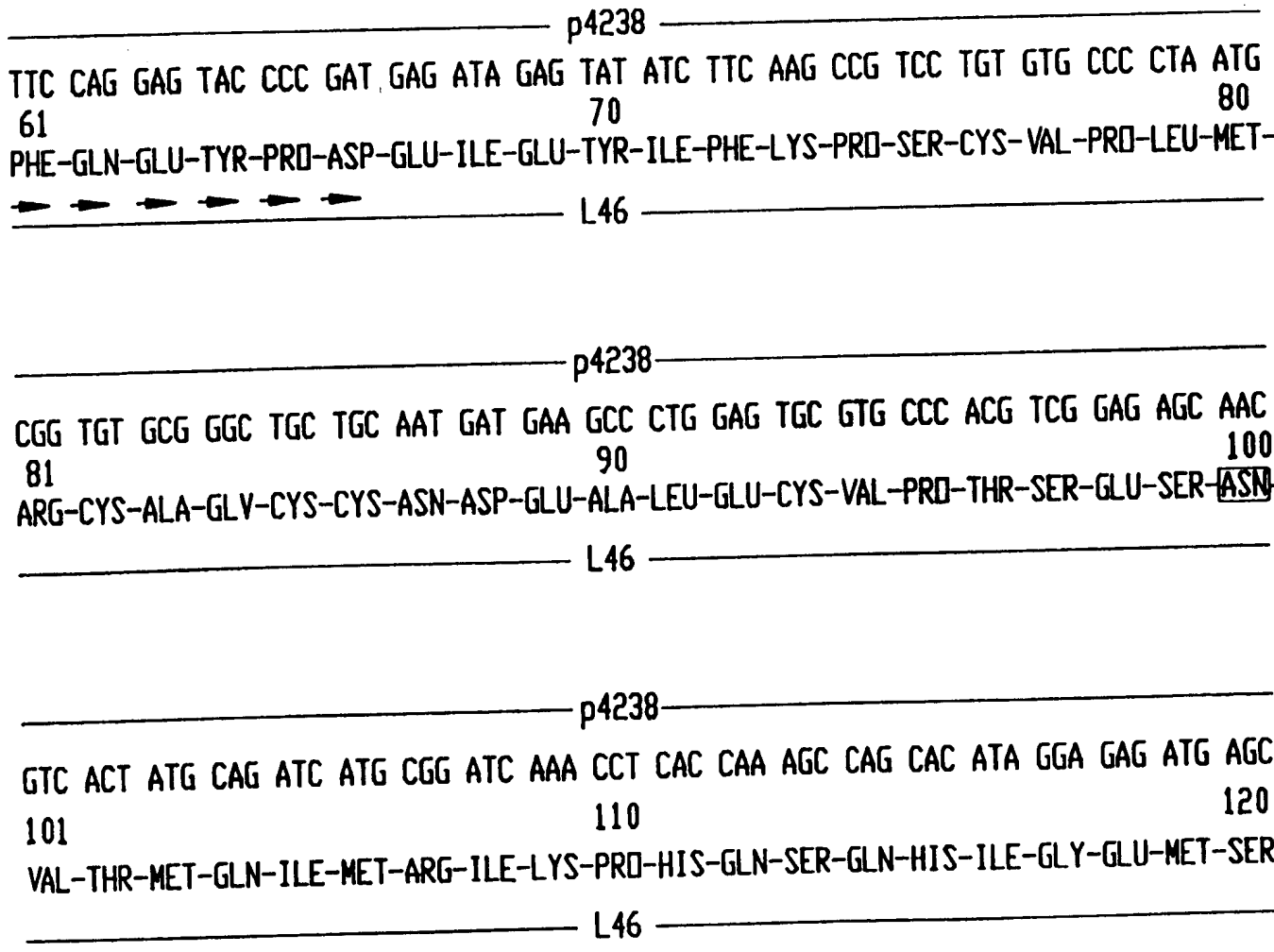
p4238
TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC TGT GTG CCC CTA ATG
61
70
80
PHE-GLN-GLU-TYR-PRO-ASP-GLU-ILE-GLU-TYR-ILE-PHE-LYS-PRO-SER-CYS-VAL-PRO-LEU-MET-
L46
p4238
CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC
81
90
100
ARG-CYS-ALA-GLV-CYS-CYS-ASN-ASP-GLU-ALA-LEU-GLU-CYS-VAL-PRO-THR-SER-GLU-SER-ASN-
L46
p4238
GTC ACT ATG CAG ATC ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC
101
110
120
VAL-THR-MET-GLN-ILE-MET-ARG-ILE-LYS-PRO-HIS-GLN-SER-GLN-HIS-ILE-GLY-GLU-MET-SER-
L46

FIG.2B

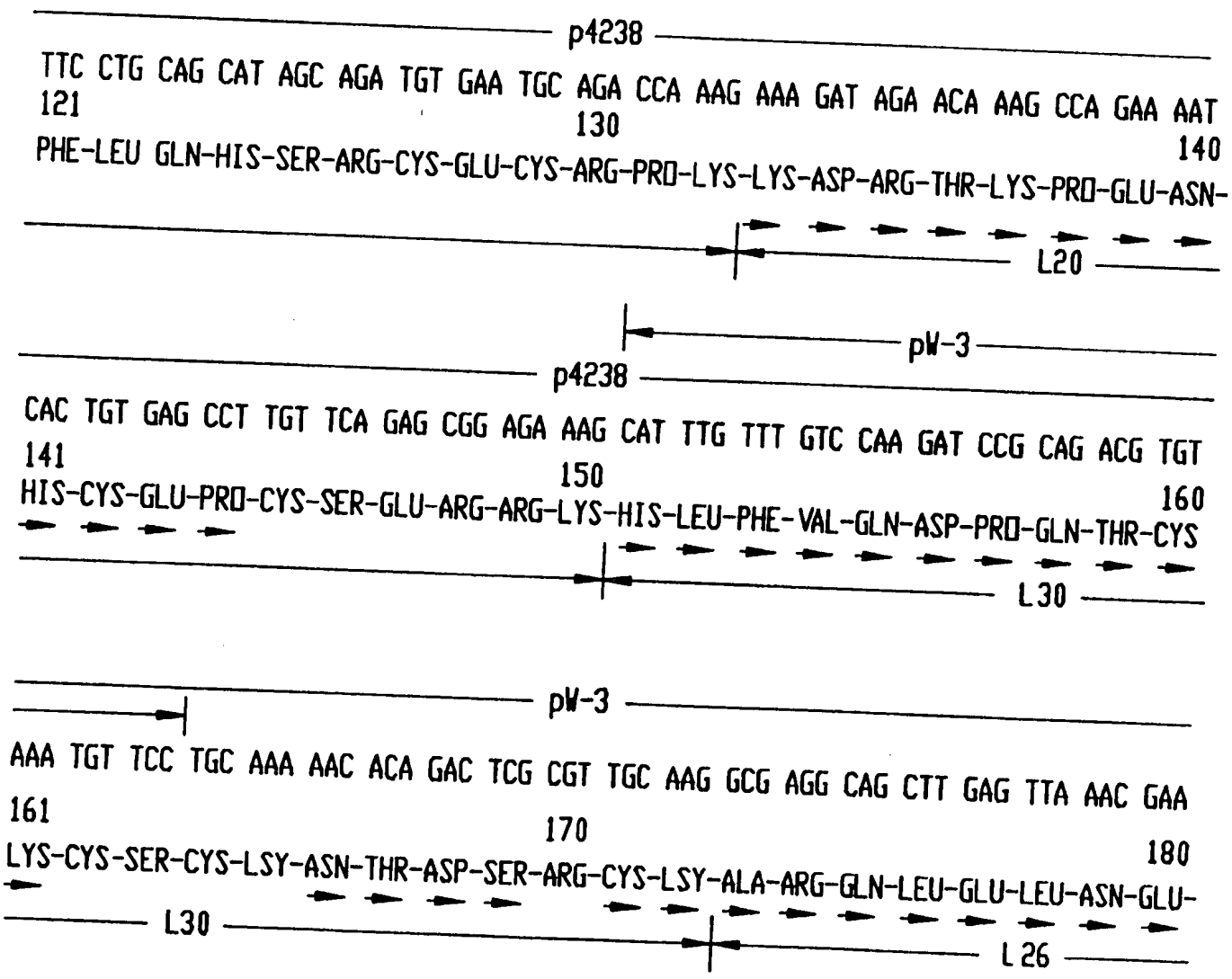
p4238
TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA ACA AAG CCA GAA AAT
121
130
140
PHE-LEU GLN-HIS-SER-ARG-CYS-GLU-CYS-ARG-PRO-LYS-LYS-ASP-ARG-THR-LYS-PRO-GLU-ASN-
L20
pW-3
p4238
CAC TGT GAG CCT TGT TCA GAG CGG AGA AAG CAT TTG TTT GTC CAA GAT CCG CAG ACG TGT
141
150
160
HIS-CYS-GLU-PRO-CYS-SER-GLU-ARG-ARG-LYS-HIS-LEU-PHE-VAL-GLN-ASP-PRO-GLN-THR-CYS
L30
pW-3
AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA
161
170
180
LYS-CYS-SER-CYS-LSY-ASN-THR-ASP-SER-ARG-CYS-LSY-ALA-ARG-GLN-LEU-GLU-LEU-ASN-GLU-
L30
L26

FIG.2C

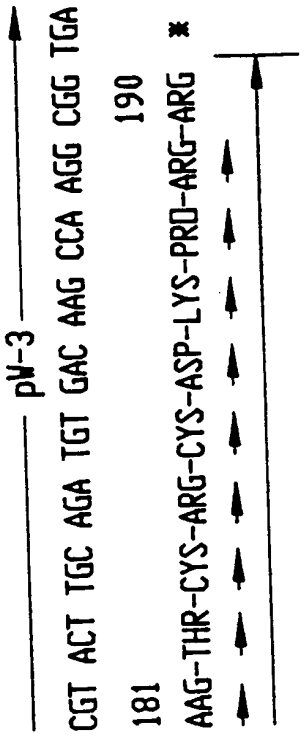
pW-3
CGT ACT TGC AGA TGT GAC AAG CCA AGG CGG TGA
181
190
AAG-THR-CYS-ARG-CYS-ASP-LYS-PRO-ARG-ARG *

FIG.2D

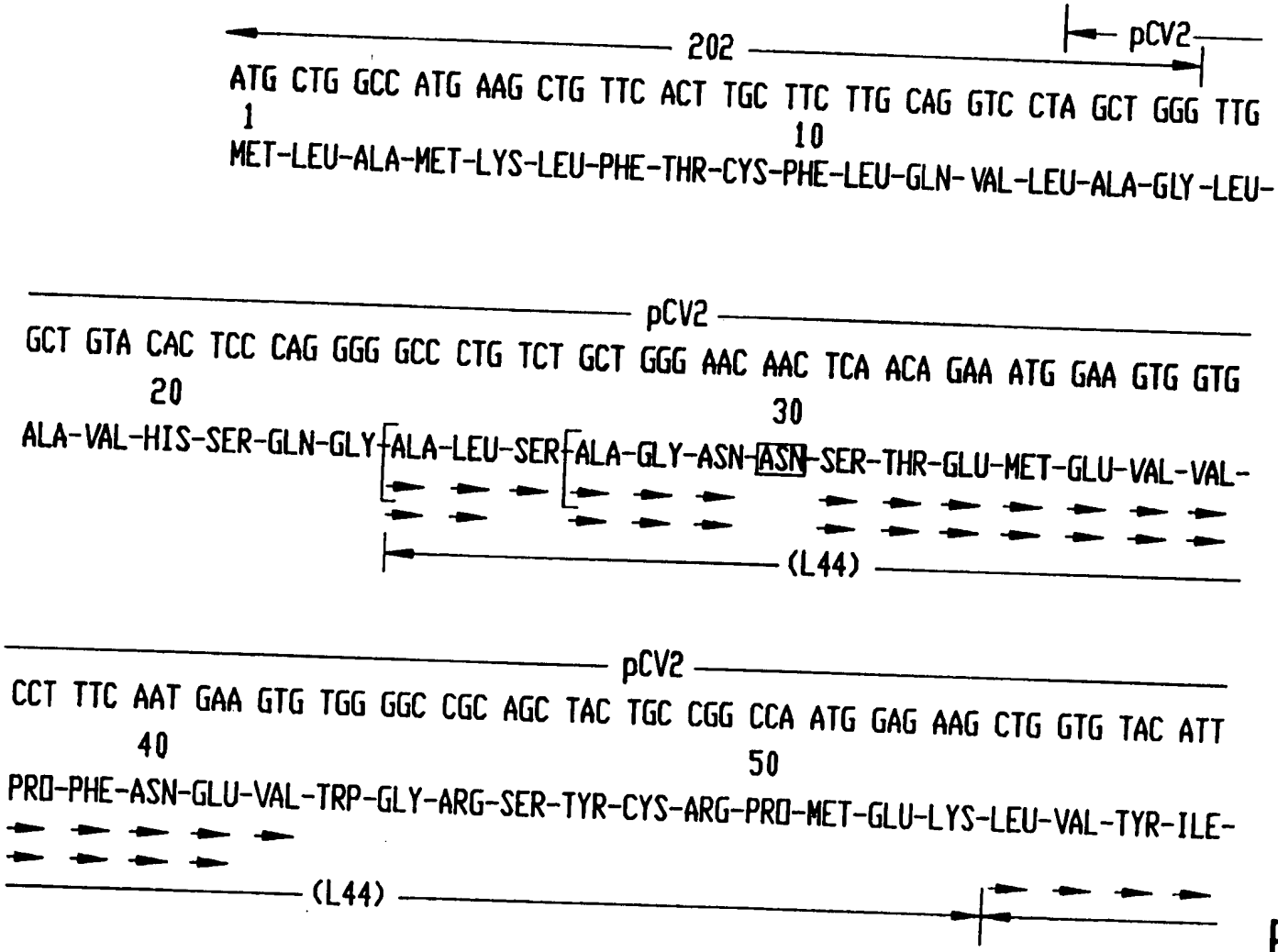

FIG.3A

EP 0 506 477 A1

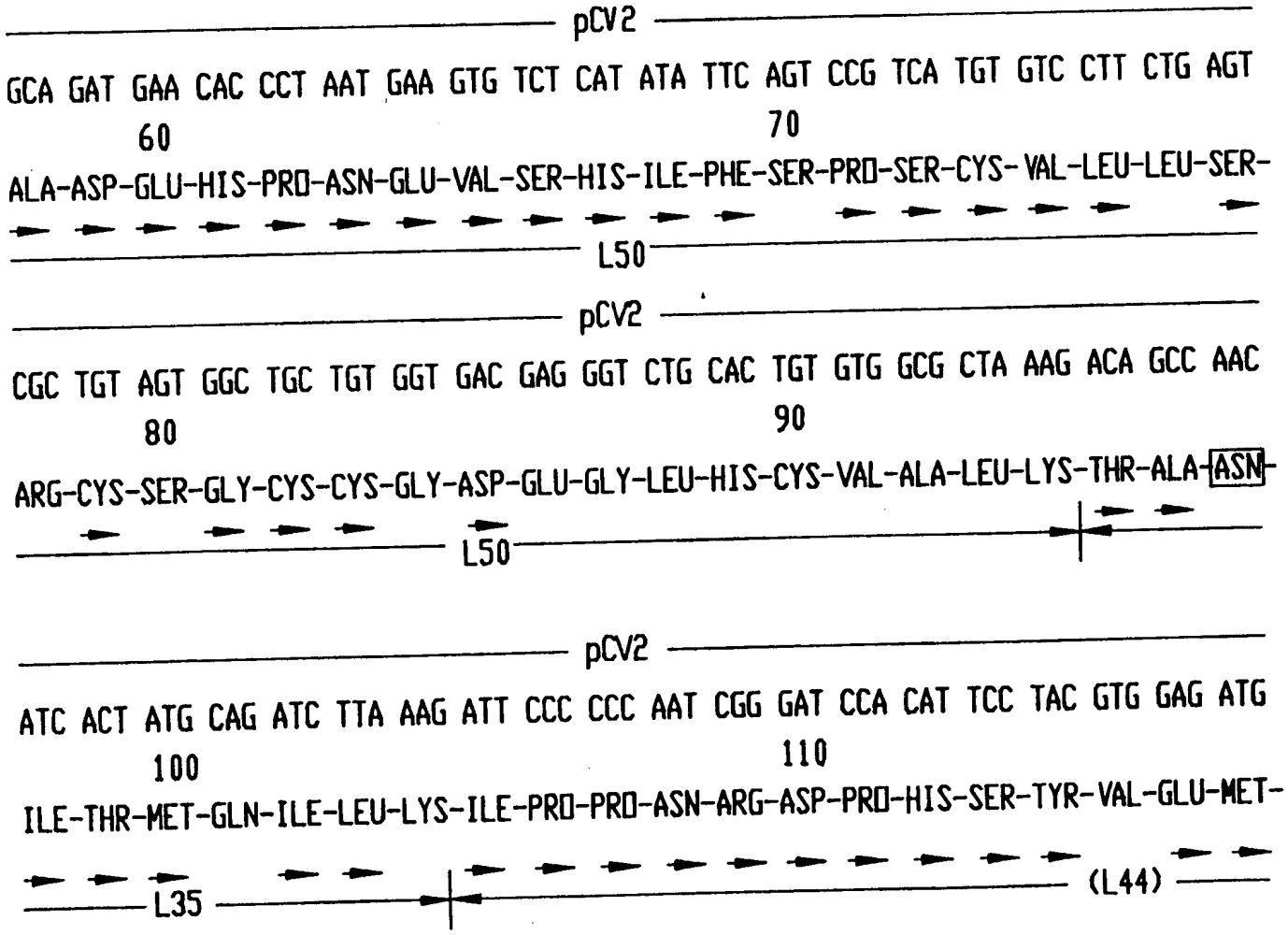
pCV2
GCA GAT GAA CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT CTG AGT
60
70
ALA-ASP-GLU-HIS-PRO-ASN-GLU-VAL-SER-HIS-ILE-PHE-SER-PRO-SER-CYS-VAL-LEU-LEU-SER-
L50
pCV2
CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT GTG GCG CTA AAG ACA GCC AAC
80
90
ARG-CYS-SER-GLY-CYS-CYS-GLY-ASP-GLU-GLY-LEU-HIS-CYS-VAL-ALA-LEU-LYS-THR-ALA-ASN-
L50
pCV2
ATC ACT ATG CAG ATC TTA AAG ATT CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG
100
110
ILE-THR-MET-GLN-ILE-LEU-LYS-ILE-PRO-PRO-ASN-ARG-ASP-PRO-HIS-SER-TYR-VAL-GLU-MET-
L35
(L44)

FIG.3B

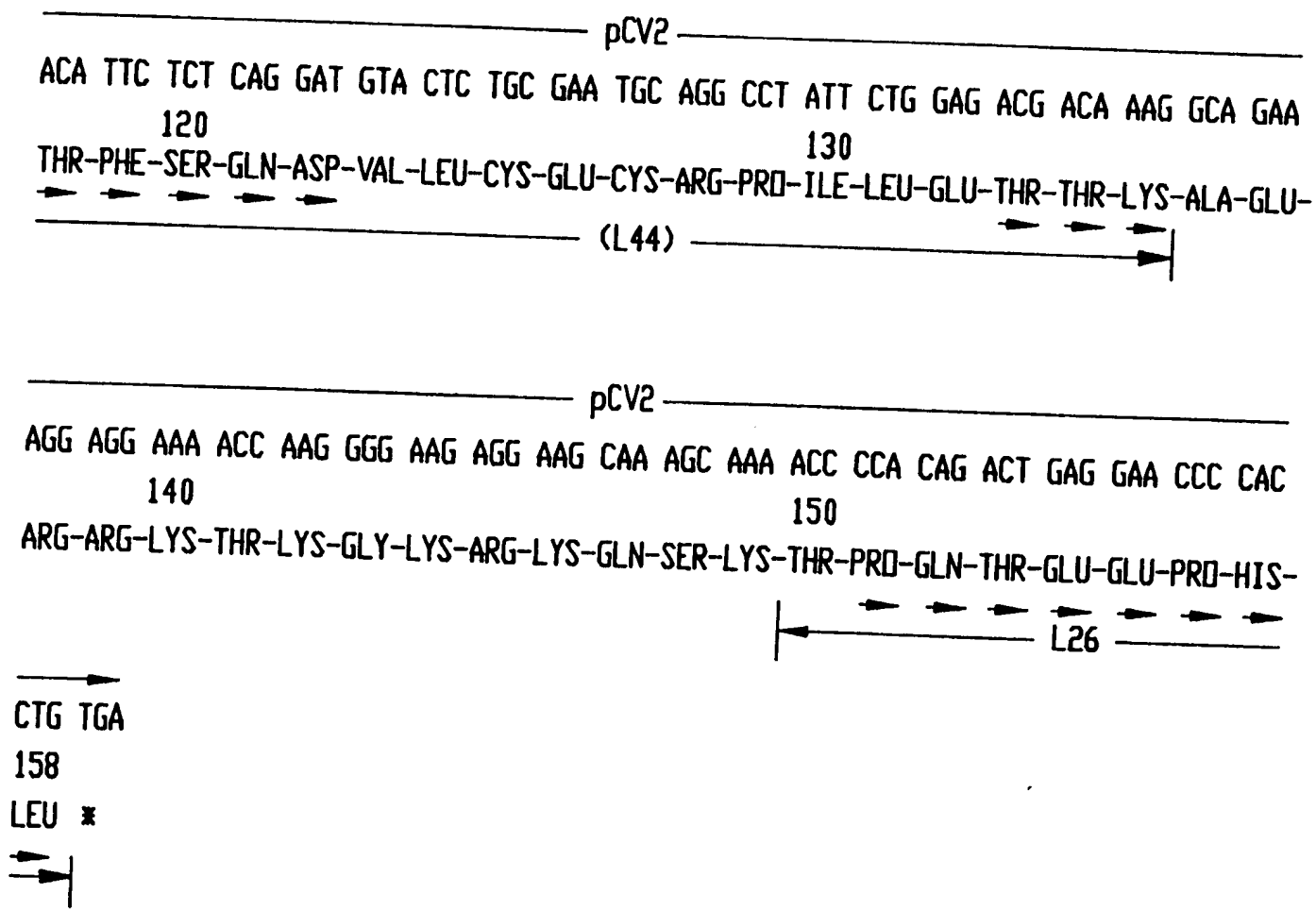

FIG.3C

EP 0 506 477 A1

```
ACCA ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG  49
     Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
                      5                  10                  15

     CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA  94
     Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
                     20                  25                  30

     GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
     Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
                     35                  40                  45

     TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
     Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
                     50                  55                  60

     TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC 229
     Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
                     65                  70                  75

     TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
     Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
                     80                  85                  90

     CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
     Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
                     95                 100                 105

     ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
     Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
                    110                 115                 120

     TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
     Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
                    125                 130                 135

     ACA AAG CCA GAA AAA TGT GAC AAG CCA AGG CGG TGA             445
     Thr Lys Pro Glu Lys Cys Asp Lys Pro Arg Arg
                    140                 145
```

FIG. 4

```
AACC ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG  49
     Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
                      5                  10                  15

     CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA  94
     Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
                     20                  25                  30

     GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
     Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
                     35                  40                  45

     TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
     Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
                     50                  55                  60

     TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC 229
     Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
                     65                  70                  75

     TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
     Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
                     80                  85                  90

     CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
     Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
                     95                 100                 105

     ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
     Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
                    110                 115                 120

     TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
     Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
                    125                 130                 135

     ACA AAG CCA GAA AAT CAC TGT GAG CCT TGT TCA GAG CGG AGA AAG 454
     Thr Lys Pro Glu Asn His Cys Glu Pro Cys Ser Glu Arg Arg Lys
                    140                 145                 150

     CAT TTG TTT GTC CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA 499
     His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys
                    155                 160                 165

     AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA 544
     Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu
                    170                 175                 180

     CGT ACT TGC AGA TGT GAC AAG CCA AGG CGG TGA                 577
     Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
                    185                 190
```

FIG. 5

```
AACC ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG  49
     Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
                      5                  10                  15

     CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA  94
     Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
                     20                  25                  30

     GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
     Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
                     35                  40                  45

     TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
     Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
                     50                  55                  60

     TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC 229
     Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
                     65                  70                  75

     TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
     Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
                     80                  85                  90

     CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
     Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
                     95                 100                 105

     ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
     Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
                    110                 115                 120

     TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
     Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
                    125                 130                 135

     ACA AAG CCA GAA AAA AAA TCA GTT CGA GGA AAG GGA AAG GGT CAA 454
     Thr Lys Pro Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln
                    140                 145                 150

     AAA CGA AAG CGC AAG AAA TCC CGG TTT AAA TCC TGG AGC GTT CAC 499
     Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His
                    155                 160                 165
```

FIG. 6A

```
TGT GAG CCT TGT TCA GAG CGG AGA AAG CAT TTG TTT GTC CAA GAT 544
Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp
                170                 175                 180

CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC 589
Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
            185                 190                 195

AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC 634
Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
        200                 205                 210

AAG CCA AGG CGG TGA                                         649
Lys Pro Arg Arg
```

FIG.6B

```
ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT      45
Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5                  10                  15

GGG TTG GCT GTG CAC TCC CAG GGG GCC CTG TCT GCT GGG AAC AAC      90
Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
            20                  25                  30

TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC     135
Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
        35                  40                  45

AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA     180
Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
    50                  55                  60

CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT     225
His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
65                  70                  75

CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT     270
Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
            80                  85                  90

GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT     315
Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
        95                  100                 105

CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT     360
Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
    110                 115                 120

CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG     405
Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
125                 130                 135

GCA GAA AGG TAA                                                417
Ala Glu Arg
```

FIG.7

```
ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT      45
Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5                  10                  15

GGG TTG GCT GTG CAC TCC CAG GGG GCC CTG TCT GCT GGG AAC AAC      90
Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
            20                  25                  30

TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC     135
Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
        35                  40                  45

AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA     180
Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
    50                  55                  60

CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT     225
His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
65                  70                  75

CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT     270
Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
            80                  85                  90

GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT     315
Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
        95                  100                 105

CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT     360
Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
    110                 115                 120

CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG     405
Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
125                 130                 135

GCA GAA AGG AGG AAA ACC AAG GGG AAG AGG AAG CAA AGC AAA ACC     450
Ala Glu Arg Arg Lys Thr Lys Gly Lys Arg Lys Gln Ser Lys Thr
            140                 145                 150

CCA CAG ACT GAG GAA CCC CAC CTG TGA                            477
Pro Gln Thr Glu Glu Pro His Leu
        155
```

FIG. 8

```
ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG GCC      45
Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala
 5                  10                  15

GGG CTG GCG CTG CCT GCT GTG CCC CCC CAG CAG TGG GCC TTG TCT      90
Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser
            20                  25                  30

GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC TTC CAG GAA     135
Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu
        35                  40                  45

GTG TGG GGC CGC AGC TAC TGC CGG GCG CTG GAG AGG CTG GTG GAC     180
Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp
    50                  55                  60

GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG CAC ATG TTC AGC CCA     225
Val Val Ser Glu Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro
65                  70                  75

TCC TGT GTC TCC CTG CTG CGC TGC ACC GGC TGC TGC GGC GAT GAG     270
Ser Cys Val Ser Leu Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu
            80                  85                  90

AAT CTG CAC TGT GTG CCG GTG GAG ACG GCC AAT GTC ACC ATG CAG     315
Asn Leu His Cys Val Pro Val Glu Thr Ala Asn Val Thr Met Gln
        95                  100                 105

CTC CTA AAG ATC CGT TCT GGG GAC CGG CCC TCC TAC GTG GAG CTG     360
Leu Leu Lys Ile Arg Ser Gly Asp Arg Pro Ser Tyr Val Glu Leu
    110                 115                 120

ACG TTC TCT CAG CAC GTT CGC TGC GAA TGC CGG CCT CTG CGG GAG     405
Thr Phe Ser Gln His Val Arg Cys Glu Cys Arg Pro Leu Arg Glu
125                 130                 135

AAG ATG AAG CCG GAA AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG     450
Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg
            140                 145                 150

AGG AGA GAG AAG TAG                                            465
Arg Arg Glu Lys
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FIG. 9

European Patent Office

EUROPEAN SEARCH REPORT

Application Number EP 92 30 2750

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A Y	EP-A-0 399 816 (MERCK) * the whole document *	1-17 18-20	C07K15/06 C12N15/19 C07K3/28 C12N5/10 A61K37/36 //C07H21/00
A	WO-A-9 102 058 (CALIFORNIA BIOTECHNOLOGY) * the whole document *	1-20	
A	WO-A-9 013 649 (GENENTECH) * the whole document *	1-20	
A	EP-A-0 370 989 (MONSANTO) * the whole document *	1-20	
P,A	EP-A-0 476 983 (MERCK) * the whole document *	1-20	
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P,Y		18-20	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C07K
C12N
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	08 JULY 1992	MOLINA GALAN E.

CATEGORY OF CITED DOCUMENTS

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